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INTERACTION BETWEEN MARINE ORGANISMS AND OIL POLLUTION

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Woods Hole Oceanographic Institution

Prepared for:

Environmental Protection Agency

May 1973

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Interaction between Marine Organisms and Oil Pollution

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Blumer, M~~ass~~; Hunt, J~~ohn~~ M.; Atema, J~~ohn~~, and Stein, L~~auren~~

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Woods Hole, Massachusetts ~~02543~~

EPA Project 18080 EBN and 18050 EBN
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Part I of this project has established that fossil hydrocarbons can be distinguished from biogenic hydrocarbons in living organisms. Hydrocarbons are stable in marine organisms and sediments and can move unaltered through several trophic levels. Only very low levels of organic stimuli are necessary for chemical communication--a mechanism especially prone to interference by pollutants.

Part II has established that a low level of crude oil (0.9 milliliters/liter) interferes with the timing of feeding behavior in the lobster (Homarus americanus). Water soluble fractions (in the 50 ppb range) did not affect feeding behavior. Added oil reduced the lipids as well as alkane and alkene-aromatic content of aquaria. Degradation of added oil followed the usual pathways of evaporation, dissolution, oxidation, polymerization, and metabolism. (EPA)

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INTERACTION BETWEEN MARINE ORGANISMS AND OIL POLLUTION

by

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ABSTRACTS

PART I

INTERACTION BETWEEN MARINE ORGANISMS AND OIL POLLUTION

The present work has established the following:

I. Hydrocarbons in uncontaminated living plants and animals differ in structure and molecular weight distribution from the hydrocarbons in fossil fuels. We have established criteria and methods that permit the detection of hydrocarbons from fossil fuels in the presence of biogenic hydrocarbons and vice versa.

II. Hydrocarbons are remarkably stable in marine sediments and in the lipids of marine organisms, even chemically reactive hydrocarbons can move unaltered through several trophic levels in the marine food web. Degradation and dispersal eventually proceeds by physical (evaporation, dissolution), by chemical (oxidation, polymerization) and by biochemical (metabolism) processes.

III. There is now ample evidence for the importance of chemical communication between marine organisms, both with inter- and intraspecific message systems. Our work shows again that only very low concentrations of organic stimuli are required for communication. Consequently, such processes appear especially prone to interference by pollutants at low concentration levels.

This report was submitted in fullfillment of project #18050 EBN under the sponsorship of the Water Quality Office, Environmental Protection Agency.

PART II

SUBLETHAL EFFECTS OF CRUDE OIL ON LOBSTER, (HOMARUS AMERICANUS) BEHAVIOR

Small quantities of crude oil (0.9 milliliters in 100 liters of seawater) interfere with some specific, possibly chemosensory, behavior of the lobster, Homarus americanus. Timing of their feeding behavior showed that the delay period between noticing food and going after it

doubles when oil was added. The water soluble fraction of this crude oil alone (in the 50 ppb range) does not have a noticeable effect on behavior and feeding times. Morphological changes in odor receptors after oil exposure were not detected by light and electron microscopy. The results indicate that small quantities of oil mixed into seawater constitute a noxious, bad smell in the lobsters environment, depressing his appetite and chemical excitability.

Chemical analyses showed that before the addition of oil a great quantity of lipids was present in the test aquaria. When the water was brought in contact with an oil slick, the lipid concentration dropped considerably. The same effect was seen in the alkane and the alkene-aromatic hydrocarbon fractions. The fate of oil in seawater followed the usual degradation pattern.

This report was submitted in fullfillment of project #18080 EBN under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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PART I

INTERACTION BETWEEN MARINE ORGANISMS AND OIL POLLUTION

Project #18050 EBN

SECTION 1

CONCLUSIONS

The present work has established the following:

1. Hydrocarbons in uncontaminated living plants and animals differ in structure and molecular weight distribution from the hydrocarbons in fossil fuels. We have established criteria and methods that permit the detection of hydrocarbons from fossil fuels in the presence of biogenic hydrocarbons and vice versa.

2. Hydrocarbons are remarkably stable in marine sediments and in the lipids of marine organisms, even chemically reactive hydrocarbons can move unaltered through several trophic levels in the marine food web. Degradation and dispersal eventually proceeds by physical (evaporation, dissolution), by chemical (oxidation, polymerization) and by biochemical (metabolism) processes.

3. There is now ample evidence for the importance of chemical communication between marine organisms, both with inter- and intraspecific message systems. Our work shows again that only very low concentrations of organic stimuli are required for communication. Consequently, such processes appear especially prone to interference by pollutants at low concentration levels.

SECTION II

RECOMMENDATIONS

1. This work has established the possibility to distinguish in environmental samples between biogenic and petroleum-derived hydrocarbons. Our baseline data come mostly from the coastal regions of the Western North Atlantic and should be extended to other coastal regions.

2. There are strong suggestions that biogenic hydrocarbons play vital roles in the life processes of marine organisms. Therefore, oil pollution may interfere with such processes at very low concentration levels. This may be a critical research area.

3. This work has shown that hydrocarbon pollutants enter the marine food web. Earlier work, based on subjective (taste) tests appears now irrelevant. The survey for oil derived hydrocarbons in fisheries products must be extended to other species and should be performed routinely on products that reach the market because of obvious public health implications.

4. No guidelines or legal limits exist for the acceptability of oil pollution in fisheries products. EPA in cooperation with public health authorities should establish such limits for the protection of the consumer.

5. The case study on the persistence of oil pollution reported here deals with a fuel oil spill. Other investigations using similar objective analytical and biological methods should be carried out with other oils and in other climatic regions.

6. Oil pollution may be more damaging through its long term and low level effects than through the gross esthetic impact. Research in the areas of low level toxicity, persistent damage, interference with chemotaxis or with reproduction has been neglected in the past. Immediate attention both to basic and applied research in these areas is needed.

SECTION III

INTRODUCTION

The goals of our study of marine pollution were summarized in our initial proposal dated May 27, 1968.

Identification of Oil Pollution

Marine hydrocarbons derive in part from living organisms and in part from pollution. We plan to establish sensitive criteria to distinguish hydrocarbons from either source. This knowledge is essential if we are to measure in the oceans the spread of the true pollutants and not of the ubiquitous natural hydrocarbons.

Fate and Persistence of Oil Pollution

Many stable pollutants, especially hydrocarbons, pass through the marine food chain with little alteration but often with a rise in concentration. We plan to study the persistence and accumulation by marine organisms, especially human food, of objectionable and hazardous compounds derived from pollution.

Effect of Pollutants on Marine Organisms

Natural compounds in the sea play an important ecological role as stimuli for the attraction of the sexes, for inter- and intraspecies recognition, in the finding of food and in short and long distance migration. Marine pollution is likely to interfere with many of these processes. Because of the extreme sensitivity of the organisms to these stimuli, such interference may be more subtle and still have a more catastrophic long-term effect than the gross toxicity of bulk pollution. We plan to study the extent to which marine pollution interferes with these processes and the concentrations which are harmful.

This work is important for pollution control through the identification of pollution and in establishing the types of compounds which have to be controlled and the levels at which they have to be held.

During the duration of this grant we have defined the natural hydrocarbon content of a wide variety of marine organisms (8, 11, 17, 19, 24) and the fate of these hydrocarbons in the marine food chain (6, 7, 8, 24). Progress during the first year of the grant period has influenced our research plan. A large coastal oil spill near Woods Hole has provided a testing ground for our concepts (6, 7, 15, 16, 22). We have succeeded

in distinguishing the spilled oil from the natural hydrocarbon background (6, 7, 16, 18, 22, 23, 24) and have shown that the spilled oil is incorporated into oysters and scallops (6, 7, 24). Thus, the first and second objectives of the initial proposal have been essentially achieved and brought to a field test. During the reporting period we have gained much new information on the environmental behavior and fate of spilled petroleum. This is relevant to the problem of oil spill identification.

Also, during the granting period we have gained experience in our research on the effect of pollutants on marine organisms. As a result, the research aims have been redefined, and instead of proceeding as initially planned for a third year, we are now planning to proceed along two separate but related lines: characterization of environmental oil samples (oil spill identification) and marine chemotaxis.

Progress in the individual areas will be discussed in the order outlined in the introduction; much of the progress is documented in publications (see list, Section XI).

SECTION IV

IDENTIFICATION OF OIL POLLUTION

Hydrocarbons of recent, biochemical origin are minor but not unimportant components of all marine organisms (8, 11, 17, 19, 21). We find a great structural variety between the hydrocarbons of different organisms. In spite of their relative complexity, the biogenic hydrocarbons are easily distinguished from those of fossil fuels. We find great differences in the structural types, in the molecular weight distribution and in the relative predominance of individual compounds. This is of obvious relevance to chemotaxonomy, to the study of chemical transormation in subsurface sediments and to the differentiation between recent hydrocarbons and fossil fuels in the environment.

We have paid special attention to:

Hydrocarbons of Marine Phytoplankton

The hydrocarbon contents of 23 species of algae (22 marine planktonic), belonging to 9 algal classes, were analyzed (17). The highly unsaturated 3,6,9,12,15,18-heneicosahexaene predominates in the Bacillariophyceae, Dinophyceae, Cryptophyceae, Haptophyceae and Euglenophyceae. Rhizosolenia setigera contains n-heneicosane, presumably derived from the hexaolefin by hydrogenation. Two isomeric heptadecenes have been isolated: the double bond is located in 5-position in the blue-green algae Synechococcus bacillaris and in 7-position in 2 green algae. In all cases, the algae contain relatively few hydrocarbons, mostly straight chain alkanes and alkenes and some monomethylalkanes. Pristane is present, but at a low concentration. Zooplankton, on the other hand, contains the complex assemblage of C_{19} and C_{20} isoprenoid alkanes and alkenes which are derived from phytol (21). The hydrocarbon composition of ancient sediments and of petroleum is far more complex; many isomeric compounds belonging to different homologous series are found, olefins are absent, and alicyclic and aromatic compounds occur at much higher concentrations than in living organisms. This work suggests that hydrocarbon analysis may be a tool for the detection of algal species in mixed plankton or in mixed algal lipids ingested by certain herbivores. Also, detailed hydrocarbon analysis enables the distinction between hydrocarbons of recent biogenic origin and hydrocarbon pollutants from fossil fuels.

Hydrocarbons of Benthic Algae

Saturated and olefinic hydrocarbons were determined in 24 species of green, brown and red benthic marine algae from the Cape Cod area (Massachusetts, USA) (19). Among the saturated hydrocarbons, n-pentadecane predominates in the brown and n-heptadecane in the red algae. A C_{17} alkylcyclopropane has been identified tentatively in Ulva lactuca and Enteromorpha compressa, two species of green algae. Mono- and diolefinic C_{15} and C_{17} hydrocarbons are common. The structures of several new C_{17}, C_{19} and C_{21} mono- to hexaolefins have been elucidated by gas chromatography, mass spectrometry and ozonolysis. In fruiting Ascophyllum nodosum, the polyunsaturated hydrocarbons occur exclusively in the reproductive structures. The rest of the plant contains n-alkanes from C_{15} to C_{21}. A link between the reproductive chemistry of benthic and planktonic algae and their olefin content is suggested.

Our analyses of the hydrocarbons in benthic marine algae from coastal environments should aid studies of the coastal food web and should enable us to distinguish between hydrocarbon pollutants and the natural hydrocarbon background in inshore waters.

An intriguing speculation is based on Paffenhofer's (1970) observation that the sex ratio of laboratory reared Calanus helgolandicus depends upon the species of algae fed to the nauplii. The percentage of males produced correlates with out analyses of heneicosahexaene in the algal food.

This and the recent discovery of an olefinic hydrocarbon acting as male attracting substance in the female gametes of a brown algae by Mueller and co-workers, suggests specific biochemical roles for hydrocarbons in marine organisms. Aside from their scientific implications these findings suggest the possibility that olefinic hydrocarbons from cracked fossil fuels might interfere with sensitive biochemical processes in the sea at concentration levels that have not before been considered potentially harmful. Further work in this area seems urgent.

Fossil Fuels, Methods

We participated in an FAO symposium on the Detection and Monitoring of Pollutants in the Marine Environment and chaired the Panel on Petroleum. A review of existing methods and a recommendation for further research has been compiled and published (21).

Characterization of the Water Soluble Fraction of Petroleum

The immediate toxicity of crude oil and oil product arises to a significant part from the water soluble fraction of the lower boiling hydrocarbons. Characterization of this fast spreading fraction is necessary and had not been carried out before. We have analyzed water extracts of crude oils and of kerosene by gas chromatography and mass spectrometry; the aqueous extracts consist predominately of aromatic hydrocarbons. Thus, in the 160-260°C boiling range substituted benzenes and naphthalenes predominate (20).

This work may aid in the identification of crude oil derived hydrocarbons in organisms and in the water column, especially at some distance from the spill and it provides the background for toxicity studies on aqueous oil extracts.

Identification of Mixed Fossil and Biogenic Hydrocarbons

The marine sediments in Buzzards Bay at West Falmouth, Massachusetts have been contaminated by a fuel oil spill in September 1969 (6, 7, 15, 16, 22). The area provides an ideal testing ground for this subject in view of the gradation between heavy contamination to clean sediment. We have established that biogenic and fuel derived hydrocarbons can be detected and determined in each others presence, even when material from one or the other source predominates strongly (22).

SECTION V

FATE AND PERSISTENCE OF OIL POLLUTION

The Environmental Ageing of Fossil Fuels

The natural alteration of biogenic and fossil hydrocarbon mixtures proceeds by physical (dissolution, evaporation), by chemical (oxidation, polymerization) and by biochemical (metabolic) processes. Different processes affect the hydrocarbon composition of a mixture in different ways and their relative role can be inferred from a chemical analysis.

We are studying the alteration of fossil fuels derived from marine spills in subtidal and intertidal sediments on the open ocean and in organisms. In the case of the subtidal sediments (22) the degradation is slow, and proceeds primarily through biochemical attack and to a lesser degree through dissolution. Oil in intertidal sediments and on the open ocean is altered primarily by evaporation and dissolution. In the case of substantial thickness (tar balls) this affects primarily the outside layer. Bacterial degradation of tar balls is negligibly slow; in beach sands it may proceed more rapidly if a sufficient supply of oxygen and of nutrients is present.

Work on this topic has begun during the last year of the present grant and will be pursued at greater intensity in the future project on Oil Spills Identification.

Hydrocarbons in the Marine Food Web

Earlier work at this laboratory had already established the persistence of biogenic hydrocarbons in the marine food web. This has been complemented with further studies both on biogenic and petroleum derived hydrocarbons.

We have now studied the occurrence of an algal derived hydrocarbon (all-cis- 3,6,9,12,15,18-heneicosahexaene) in marine animals (8). This olefin is accumulated nonselectively by Rhincalanus nasutus from its algal food together with the triglyceride lipids. Other related copepods and other zooplankton species contain little or none of this hydrocarbon, even when grown in cultures of algae that provide R. nasutus with that olefin. The presence of this hydrocarbon in marine vertebrates shows that mechanisms for its injection into higher trophic levels, either via R. nasutus or other unidentified vectors, exist and that even such a highly unsaturated and unstable hydrocarbon is stabilized within animal lipids.

The Oil Spill of West Falmouth, Massachusetts

This incident has been described in published and unpublished reports (6, 7, 15, 16, 22). The essential conclusions regarding persistence, spread and biological effect of this oil spill remain the same. Oil is still chemically identifiable and the original animal populations have not returned except at the most lightly polluted marginal locations.

Biochemical degradation of the oil has accelerated during summer 1970 and the straight chain hydrocarbons have almost completely disappeared at some locations. Branched and cyclic hydrocarbons persist and the original boiling point distribution of the oil has been preserved remarkably well (22).

Of special interest are the analyses at an offshore, subtidal station at a considerable distance from shore. At this station oil from the spill did not appear in the sediments until at least seven months after the accident. During the following months the oil content of the sediment increased further and by September 1970, one year after the spill, the contamination reached a level similar to that in the originally more polluted stations closer to shore (22).

Because of the more rapid bacterial degradation of normal paraffins, ratios of normal to branched paraffins of similar boiling point are a sensitive measure of the relative degree of biodegradation of an oil. In the sediments at West Falmouth, this parameter demonstrates the gradual environmental modification of the oil. Remarkably, at all stations the degree of degradation is halted and reversed for one to several months during spring to early summer of 1970. This, and the late appearance of oil at the offshore station discussed above strongly suggest the movement of a less degraded oil from the same spill from shore into the sediments of the open Buzzards Bay, most likely as a result of sediment movement.

SECTION VI

MARINE CHEMOTAXIS

Some natural organic compounds are of great importance to marine organisms serving as signals conveying information about the environment. Organisms are extremely sensitive to the presence of such stimuli. Because of this sensitivity and the very low concentration level at which the natural stimuli occur in the sea, pollution may interfere with the natural chemotaxis of organisms in a subtle but potentially catastrophic way. Our research in this area is multidisciplinary and integrates chemical studies with those in animal behavior and neurophysiology.

The principal objectives of the program are:

1. Survey of marine animals to determine the extent of their dependence on chemical communication.

2. Development of sensitive bioassays for testing chemicals involved in animal communication and for the detection of the interference by pollutants with natural communication.

3. Isolation and structure determination of natural communicants, synthesis of model compounds and studies of the relationship between structure and activity.

The eventual goal is an understanding of the response of marine animals to chemical stimuli; this will enable us to predict the effect of a pollutant of a given structure on chemical communication in the ocean.

In our initial proposal to FWPCA we requested funds to initiate a program to investigate the effects of pollution on marine chemotaxis. The effort is now well underway and our effectiveness has been strengthened through the hiring - through independent funding, of a neurophysiologist and an animal behavior specialist. They perform a detailed survey on the importance of chemotaxis to marine fishes. Methods include anatomical studies of brain and sensory centers, behavior studies and neurophysiological investigations of organisms in miniature ecosystems.

Two chemical communication systems are being investigated in detail. The first involves the attraction of starfish to oyster extracts; the second involves the attraction of a flatworm to its host, the horseshoe crab. In both cases the chemicals are water soluble, have low molecular weights and are active in the part/per billion range.

Starfish Chemotaxis

This work, introduced in earlier summaries, has been pursued in two directions.

The starfish Asterias vulgaris was observed in aquaria and in a flow tank in the presence of oysters, oyster tissue, and extracts of four shellfish species (10). Oysters were approached after varying delays, opened, and consumed; oyster tissue was rapidly approached and eaten. Dilute (ppb) shellfish extracts were appraoched in a flow tank. High concentrations of extracts, elicit posturing and stomach eversion responses. This phase of our investigation was aimed at an understanding of the behavior of starfish in the presence of a chemical stimulus and in the hope of the eventual isolation and identification of the components of the stimulus system.

In addition, we noted during our investigation erratically high responses to certain blank test solutions. These were traced to the presence of compounds attractive to starfish in the perspiration of the experimenter. Several chemicals known to occur in human perspiration have now been demonstrated to elicit an approach and feeding response in starfish.

This unexpected finding again emphasizes the need for great caution in planning and performing chemotaxis studies; in addition, it has given us insight into the range of compounds which are sensed by Asterias vulgaris.

Chemotaxis in the Symbiosis of Limulus and Bdelloura

The host specific substances released by the horseshoe crab Limulus polyphemus, attracting the symbiotic flatworm Bdelloura candida have been investigated. Water from horseshoe crab tanks contains metabolites which are attractive to the flatworm, whereas water from other crustaceans has no effect. We have investigated and isolated two active compounds that act together as stimulus. One has been identified as trimethylamine hydrochloride, the other is still unidentifed. The response of Bdelloura to synthetic organic compounds has been investigated. Ammonium acetate, zinc chloride, dieldrin and aqueous kerosene extracts had no immediately apparent effects on the response of Bdelloura to Limulus at 100 ppm. On the other hand, iron chloride, phosphates (typical of those in household detergents), detergents and mercuric chloride interfered with the response of Bdelloura to Limulus water at 100 ppm but not at 1 ppm.

Exploratory Studies on Chemotaxis in Lobster and Alewife

With increasing experience in work with lower and relatively unsophisticated animals (Asterias, Bdelloura) we have proceeded to exploratory work on higher organisms that are also more important commercially.

a). The effects of kerosene and kerosene fractions on the behavior of lobster Homarus americanus have been investigated. Kerosene was fractionated into three fractions; straight chain hydrocarbons, branched/cyclic hydrocarbons and aromatic hydrocarbons (water soluble part of kerosene). Before the test the animals were observed until their normal behavior was classified into 65 behavioral units, such as attack, grooming, tail flips, etc. Kerosene caused an increase in stress behavior, a high degree of grooming, stimulation of feeding and an increase in aggression. The aromatic hydrocarbon fraction caused the same responses as those observed in the kerosene test except that feeding behavior was inhibited, although attraction to the stimulus was still evident. The branched/cyclic hydrocarbon fraction caused only weak attraction and stimulation of feeding. The straight chain hydrocarbons had no observable effect on behavior.

b). The recognition of the homestream by alewives (Alosa pseudoharengus) returning to their spawning ground has been demonstrated to involve the sensing of chemical stimuli that are specific to the homestream water. We find that the active components in this "chemical fingerprint" are heat stable, nonvolatile, polar and of a molecular weight below 1,000. Tests on processed fractions from the homestream suggest that acids and bases are involved but no lipids and inorganic anions and cations.

We hope that continuation of this work will aid in predicting the danger of pollution with certain chemicals to the homestream recognition of commercially important fishes such as the alewife and especially the salmon.

Conclusions from Chemotaxis Studies

These and other investigations in this field demonstrate the extent to which chemotaxis mediates many important life processes of marine organisms. The recent investigations by Mueller on chemotaxis in brown algae have shown that hydrocarbons, previously thought to be relatively inert byproducts of plant and animal metabolism, are directly responsible for the attraction of male gametes to the egg cell. Similarly, our work on the hydrocarbon composition of marine benthic and planktonic algae suggests that hydrocarbons may be in-

timately involved in cell division or reproduction.

Obviously, chemotactic processes occur at the molecular level and conversely, they can be interfered with by chemicals at the molecular level. We have carried out exploratory work on the attraction of the mud snail Nassarius obsoletus to oyster extracts. This response is depressed by minute amounts of aqueous kerosene extracts. (A saturated kerosene extract after a dilution by 10^{10} still depresses the food response by as much as 40%). This suggests that pollution may be ecologically damaging at concentration levels far below those normally considered harmful.

Our investigations directly related to chemotaxis have been largely exploratory; however, we have learned much about the complexity of the processes involved in stimulus recognition, especially in higher animals and about the necessary precautions that have to be taken in experimentation.

We feel that it is important to continue this phase of the investigation because of its implications.

SECTION VII

ACKNOWLEDGEMENTS

We wish to thank Dr. C. S. Hegre, Project Officer, for his support, advice and concern.

SECTION VIII

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SECTION IX

PUBLICATIONS

Listed are all publications completed with partial or full support of Grant 18050 EBN.

1. Blumer, M., Marine Hydrocarbons from Organisms and Pollution. Abstract, A.C.S. Meeting, September 1970, Chicago, Ill. WATR 50.

2. Blumer, M., Oil Contamination and the Living Resources of the Sea. A Review - presented at the FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing. Rome, December 9-18, 1970.

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4. Blumer, M., On the Impact of Oil Port and Refinery Operations on the Coastal Ecology and Food Derived from the Sea. Testimony. In Hearings before the Subcommittee on Air and Water Pollution of the Committee on Public Works, United States Senate, Ninety-first Congress, Second Session, Machiasport, Maine, Sept. 8 and 9, 1970. p. 33-42.

5. Blumer, M., On the Environmental Effects of Increased Oil Traffic in the Potomac. Testimony before the Conservation and Natural Resources Subcommittee. Washington, July 22, 1970.

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Consultation, Testimony and Analyses for Government Agencies

We have advised Dr. T. Murphy, Federal Water Quality Administration, prior to a hearing at the Department of the Interior.

We have advised Mr. E.R. Baird, U.S. Army Engineers, Norfolk District, on oil pollution, in connection with a law suit prepared by the Corps of Engineers.

We have served on a FWPCA-API panel on the toxicity of dispersants and have also served on a U.S. Coast Guard - National Academy of Sciences Panel on Pollution Monitoring, and on a FWQA Panel in Bacterial Degradation of Oil Pollution.

Further, we have testified at Government request:

On the Ecological Risks Inherent in Expanded Offshore Drilling and Oil Transport: to the Subcommittee on the Judiciary, U.S. Senate.

On the Impact of Oil Port and Refinery Operations on the Coastal Ecology and Food Derived from the Sea: to the Subcommittee on Air and Water Pollution of the Committee on Public Works, U.S. Senate.

On the Environmental Effects of Increased Oil Traffic in the Potomac: to the Conservation and Natural Resources Subcommittee.

We have served as a member of the U.S. Delegation to the NATO/ CCMS Ocean Oil Spills Conference, Brussels, November 1970.

We have presented testimony at the request of the Maine Environmental Improvement Commission.

At the request of EPA, we have carried out analyses of shellfish speciments for the possible presence of petroleum derived hydrocarbons.

We have carried out analyses regarding shellfish pollution and beach pollution for the Town of Falmouth, Mass., for the Department of Public Health, Commonwealth of Massachusetts and for the Martha's Vineyard Conservation Society.

Dr. Blumer was a member of the Pollution Committee, Town of Falmouth, Massachusetts, 1970-1971.

SECTION X

APPENDIX

Benthic Algae

Class	Order	Species	Date (1970)/Collection Station[a]
Chlorophyceae	Ulotrichales	Enteromorpha compressa	July/2
		Ulva lactuca	25 May/1
	Cladophorales	Spongomorpha arcta	25 May/1
	Siphonales	Codium fragile ssp. Tomentosoides	July/2
Phaeophyceae	Ectocarpales	Ectocarpus fasciculatus	25 May/1
		Pilayella littoralis	25 May/1
	Chordariales	Chordaria flagelliformis	25 May/1
		Leathesia difformis	1 June/3
	Punctariales	Punctaria latifolia	1
		Scytosiphon lomentaria	1 June/3
	Laminariales	Chorda filum	1 June/3
		Chorda tomentosa	25 May/1
		Laminaria agardhii	14 June/1
		Laminaria digitata	25 May/1
	Fucales	Ascophyllum nodosum	25 May/1
		Fucus distichus ssp. Edentatus	25 May/1
		Fucus spiralis	25 May/1
		Fucus vesiculosus	25 May/1
Rhodophyceae	Bangiales	Porphyra leucosticta	25 May/1
	Cryptonemiales	Dumontia incrassata	25 May/1
	Gigartinales	Chondrus crispus	25 May/1
	Rhodymeniales	Rhodymenia palmata	25 May/1
	Ceramiales	Ceramium rubrum	25 May/1

Stations: 1 Sandwich Jetty, Barnstable, Mass.; 2 Little River, Waquoit Bay, Mass.; 3 West Falmouth Harbor Jetty, West Falmouth, Mass.

Planktonic Algae

Bacillariophyceae	
	Cyclotella nana (3H)
	Ditylum brightwellii (D. Bright.)
	Lauderia borealis (Clone 14)
	Rhizosolenia setigera (Rhizo)
	Skeletonema costatum (Skel)
	Thalassiosira fluviatilis (Actin)
	Thalassiosira sp.
Dinophyceae	
	Gonyaulax polyedra (GP 60e)
	Gymnodinium splendens (Gym. s.)
	Peridinium trochoideum (Peri)
	Peridinium trochoideum, old culture
Cryptophyceae	
	Cryptomonas (Rhodomonas?) sp. (3C)[a]
	Cryptomonas (Rhodomonas?) old culture
Haptophyceae	
	Coccolithus huxleyi (BT-6)
	Isochrysis galbana (Iso)
	Pheaocystis poucheti (Pp)
	Pheaocystis poucheti (677-3)
Euglenophyceae	
	Eutrepiella sp. (W. H. Eut.)
Cyanophyceae	
	Oscillatoria woronichinii (Sm 24)
	Synechococcus bacillaris (Syn.)
Rhodophyceae	
	Porphyridium sp. (Porph)
Xanthophyceae	
	Tribonema aequale (No. 50)[b]
	Undetermined sp. (GSB Stiocho)[c]
Chlorophyceae	
	Dunaliella tertiolecta (Dun.)
	Derbesia tenuissima (LB 1260)

a Now believed to be a species of Chroomonas.

b A freshwater algae, studied because of its clear taxonomic position. No certain marine xanthophytes are available.

c Systematic position uncertain. May belong to the new class Eustigmatophyceae.

Copepods

Rhincalanus nasutus
Eucalanus bungii californicus
Calanus helgolandicus (pacificus)

Shellfish

Crassostrea virginica
Aequipecten irradians

Other Animals

Asterias vulgaris
Limulus polyphemus
Bdelloura candida
Homarus americanus
Also pseudoharengus
Nassarius obsoletus

PART II

SUBLETHAL EFFECTS OF CRUDE OIL ON LOBSTER,

(HOMARUS AMERICANUS) BEHAVIOR

Project #18080 EBN

SECTION I

CONCLUSIONS

1. Effects on behavior and feeding times of the lobster Homarus americanus were seen only in the first experiment when crude oil was directly added to their aquaria (ration oil-sea water = 1:100,000). No effects could be measured in the second experiment when only the soluble fraction of this crude oil was added. In the latter case, oil was added to the water in the same amount (1:100,000) and the soluble fraction was recovered from the sea water in the range of 50 parts per billion (ppb).

2. The effects on behavior are interpreted as changes in water chemistry sensing movements. The change in feeding time was caused by a doubling of the waiting phase, which defines the time period in which the lobster has noticed the food but has not yet left his shelter to search for it.

3. Light and electron microscopy showed no observable changes in morphology of odor receptors. The results are therefore interpreted to be caused by the depressing quality of crude oil: a "bad odor" effect.

4. In the experiment on effects of solubles, some petroleum hydrocarbons among a large amount of lipids were present in the aquaria before oil was added. After addition of oil, both lipids and hydrocarbon concentrations went down considerably. The degrading oil showed a different composition each day in both experiments (crudes and solubles), in general following the usual pattern.

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SECTION II

RECOMMENDATIONS

It can be seen from the results of this study that levels of oil in sea water can be determined which could be allowable for lobsters and other marine animals. Using our techniques, sublethal effects can be observed and quantified.

1. In an expanded effort different (higher) levels of oil should be tested.

2. Other animals should be tested in a similar fashion.

3. The experiments should be repeated under natural conditions.

With the addition of the 3 experiments recommended above, reasonable water quality standards can be set regarding petroleum hydrocarbons in the sea.

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SECTION III

INTRODUCTION

It is well known that oil can kill marine animals, affecting both inhabitants of surface waters and bottom dwellers for long periods of time (Blumer, 1970). It is also becoming increasingly clear that marine organisms depend on chemical communication in one way or another for individual and species survival. For example, catfish cannot locate food without their sense of taste (Atema, 1971) and lobsters utilize a sex attractant for mating (Atema and Engstrom, 1971). The possibility of interference with chemical communication by extremely low levels of crude oil has been pointed out (Blumer, 1970). Oil is not only present as a surface film or in bottom sediments; several fractions also exist in solution and emulsion in considerable quantities (Boylan and Tripp, 1971). With the increasing chance of coastal oil spills and the understandable public confusion on ill effects of oil on lobster populations in mind, we have tried to develop methods of determining sublethal effects of crude oil on lobsters under carefully controlled conditions. The results of such a study on a much larger scale could be used to determine what level of oil pollution becomes intolerable for marine life and might also indicate what physiological mechanisms are involved in the effects of oil on the behavior of these animals.

Oil is a complex stimulus; its effects on behavior are difficult to measure because many hydrocarbon fractions have very different properties. After various pilot studies on effects of oil and kerosine fractions, we decided to use whole crude oil and its water soluble fraction. We selected from the lobsters' behavior the most reproducible pattern; feeding behavior, as a standard in which to measure sublethal oil effects.

As a measure for physiological damage, we chose to examine the microscopical and submicroscopical structures of the aesthetasc hairs on the antennules. These appendages serve an olfactory function and are, together with the gills, probably the most sensitive membranes to the outside environment.

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SECTION IV

METHODS

A. Keeping of Animals and Test Procedures

Sixteen mature lobsters (Homarus americanus) caught locally around Woods Hole, Massachusetts, were used in the experiments. Carapace lengths (measured from the rear of the eye socket to the beginning of the abdomen) ranged from 6.5 cm to 10.1 cm with most lobsters measuring around 9 cm. Animals that had molted one to three months prior to the start of the experiment were chosen to exclude molting and premolt effects during the experiment.

All the animals were housed in individual 100 liter fiberglass tanks with one glass window for observation. Tanks were maintained at approximately 22°C in a closed system. A large airstone in each tank provided aeration and circulation of the water. All animals were given at least two weeks acclimation time, prior to the beginning of the experiments, during which they were starved. Dim room light and a diurnal schedule were maintained throughout.

During the experiment, each animal was observed individually in its behavior just before and after the introduction of food. The lobsters were observed each day between 0800 and 1200 hours for ten consecutive days. The order of testing individuals was constant, so that each lobster was tested at the same time each day. A single observation session for each animal consisted of two parts: (1) a ten minute period, during which all behavior units were recorded; at the end food was introduced; (2) the timing of the lobster's feeding behavior.

Food, a small piece of fresh mussel, Mytilus edulis, was slowly lowered on nylon string at the opposite side of the tank to where the lobster was situated. In order to do this two pieces of food were always available, one on the left and one on the right side of the tank. The introduction method did not elicit any immediate behavioral responses attributable to visual or mechanical stimuli. On this amount of food the animals were always kept hungry (McLeese, 1972).

Three periods of the feeding behavior sequence were then timed: 1) the alerting phase; from introduction of food to alert, 2) the waiting phase; from alert to the beginning of search, 3) the searching phase; from the beginning of search to hit. Alert was determined by an increase in antennule and/or exopodite rate while a general body movement marked the beginning of search. Hit was the moment of first physical contact with the food by the feeding appendages.

Two series of experiments were done, one with whole crude oil and one with the water soluble fraction of crude oil. In the whole oil experiment eight lobsters, four males and four females were used. All eight were selected and treated simultaneously. In the experiments on the effects of the water soluble fraction four male lobsters were tested at one time, followed by an identical series of four females. Oil introduction methods are reported later.

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The crude oil tests were done in the summer of 1971, the tests with the water soluble fraction were done in the winter of 1972. In both experiments one animal showed aberrant behavior and was eliminated. The tests were done with seven animals for crude oil and seven animals for water soluble fraction.

B. Behavior Recording and Analysis

1. Behavior recordings were made in shorthand using behavior description units (see Appendix Table I), which we developed in the course of our work on lobster behavior. The shorthand code was transcribed on data code forms for analysis of the frequency with which each unit appeared in each animal during each daily observation period. Superficial judgment of the frequency scores resulted in elimination of several units and grouping of others. The units that did not occur frequently enough to allow statistical treatment were eliminated. The units "Rake 1, 2, 3" were grouped as one: "Rake". The five groom units were grouped as "Groom". Table I lists the resulting units which were analyzed.

Of these units, three required further treatment before statistical analysis. Two of those, "Beat" and "Fan", describe appendage movement rates in three categories: "Slow, Medium and Fast". The third describes "Claw Position" in "1, 2 and 3". Since these units must occur in one of the three categories all the time, only their relative occurrence is of importance. With our recording procedure it is not possible to measure duration of each behavior unit. For example, an approximation was obtained by calculating the number of "Fast" rates as a fraction (in percent) of the total number of "Beat". For instance, if for one animal during one observation period "Beat" was recorded 10 times, of which two were Fast, three Medium and five Normal, the relative occurrence was 20%, 30% and 50% respectively. These normalized numbers (20, 30 50) were then analyzed as all other units (Appendix Table II, a and b).

Analysis of the duration of feeding behavior phases were done as for behavior units. The time in seconds was listed for "Alert", "Wait" and "Search" for each animal and each day (Appendix Table III).

2. Statistical analysis of these behavior data is made difficult by great individual differences between such complex animals as lobsters. To allow for individual differences a non-parametric technique was used to test the significance of changes in unit frequencies. Treatment of all units, including normalized units and duration times was identical. A sample treatment will be given for the unit "Crude, Antenna Wave"*.

Table II lists the 10 experimental days (horizontal) and the seven individual animals (vertical). The numbers in the table represent the frequency of occurrence of the behavior unit "Crude, Antenna Wave" over a ten minute period per animal per day. In other words, each number means how many times one lobster waved his antennae in the ten observation minutes of that particular day. A x^2 test was used to determine whether or not the unit frequency changed significantly from the first five days (before oil) to the next five days (after oil). For this, the two highest scores and the

* The units are preceded by "Crude" or "Soluble" to denote the experiments

TABLE I

SELECTED BEHAVIOR UNITS USED IN ANALYSIS

Name		Definition
1. Antenna wave		slow sweep of an antenna
2. Antennule wave		change in antennule positior
3. Wipe		wiping of the antennule by the third maxillipeds
4. Rake		back and forth movement of one or more walking legs (pereiopods) across the substrate while body is still
5. Scoop		lifting of walking legs from substrate to mouth
6. Move		any undirected movement of the body
7. Climb		raising of body against the side of the wall to a vertical position
8. Groom		picking, rubbing, scratching parts of the body with the walking legs
9. Antennule:	Beat Slow	beating of the antennules at a slow or normal rate (0-60 beats per minute)
	Beat Moderate	beating at a moderate rate (60-120 bpm)
	Beat Fast	beating at a fast rate ($\geq$ 120 bpm)
10. Fan:	Slow	slow fanning of exopodite of 3rd maxilliped (90 bpm)
	Moderate	moderately fast fanning (90-180 bpm)
	Fast	fast fanning ($\geq$ 180 bpm)
11. Claw 1		claws closed
Claw 2		claws half open
Claw 3		claws wide open

TABLE II

FREQUENCY OF OCCURRENCE OF BEHAVIOR UNIT "CRUDE, ANTENNA WAVE"

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	4	5	2	2	5	14	14	4	16	10
2	16	14	5	3	19	8	28	29	29	7
3	8	9	3	1	22	26	22	36	32	29
4	5	3	1	6	11	17	7	9	9	3
5	14	9	0	1	2	10	1	5	0	1
6	2	8	0	0	6	10	14	6	6	1
7	10	8	2	5	18	2	5	20	9	12

two lowest scores were considered. In case of a tie all tied numbers in that animal were taken, until at least two numbers of both extremes were represented. In cases of frequent zero scores (for instance see Appendix Table IIa, "Crude, Scoop") this procedure resulted in large numbers of low scores.

C. Introduction of Crude Oil

A volume of 0.9 ml of La Rosa crude oil was introduced on to the surface water of each test aquarium on day six at 9 a.m. This represents an oil-water ratio of 1:100,000. A uniform thin brown slick soon covered almost the entire surface of the aquaria. Vigorous airstone bubbling resulted in some of the oil adhering to the wall of the aquaria near the airstone. Emulsification undoubtedly took place from the beginning (Boylan and Tripp, 1971). Near the end of the five day period the surface film had disappeared. Only small brown particles floated at the surface. Many such particles were stuck to the walls.

After completion of the ten day experiment the antennules of the experimental animals were removed and prepared for electron microscopical examination.

D. Introduction of Water Soluble Fraction of Crude Oil

In order to expose the test animals exclusively to the water soluble fraction of crude oil a 100 liter all glass mixing tank was set up separate from the test aquaria housing lobsters (see Figure 1). Water was pumped from near the bottom of the glass mixing tank into four test aquaria using glass tubing, teflon connectors and a peristaltic pump (Manostat standard model). Pumping rate was one gallon per minute. The four aquaria received equal amounts of water and fed the extra volume back to the mixing tank by gravity through glass tubing and a silicone rubber end piece to minimize vandalism by the lobsters. The outflow of the feedback was near the bottom of the oil slick tank, away from the intake to insure good mixing. In the first five days, no oil was present in the system. On day six, 4.5 ml of La Rosa crude oil was introduced on the surface of the mixing tank. This volume was calculated to result in an oil-water ratio of 1:100,000, similar to the ratio used in the whole oil experiment. A variable speed electric mixer (Eastern model 5 VA) was used at a constant low speed (200 rpm) to insure proper circulation of water under the oil film without causing an emulsion (Boylan and Tripp, 1971).

This series was completed for four male lobsters and repeated with four female lobsters, one of which was eliminated as mentioned earlier.

E. Photographic Record

Each day after the biological observations, black and white photographs were taken of the slick in the mixing tank to follow its visual appearance.

F. Determination of Hydrocarbons and Lipids in Oil-Sea Water Mixture

1. Sampling and Extraction

To collect samples of the whole oil-water mixture for chemical analysis a separate aquarium was set up, identical with the others, including airstones, lobster size and feeding. No observations were recorded on this lobster's behavior. Crude oil (0.9 ml La Rosa crude)

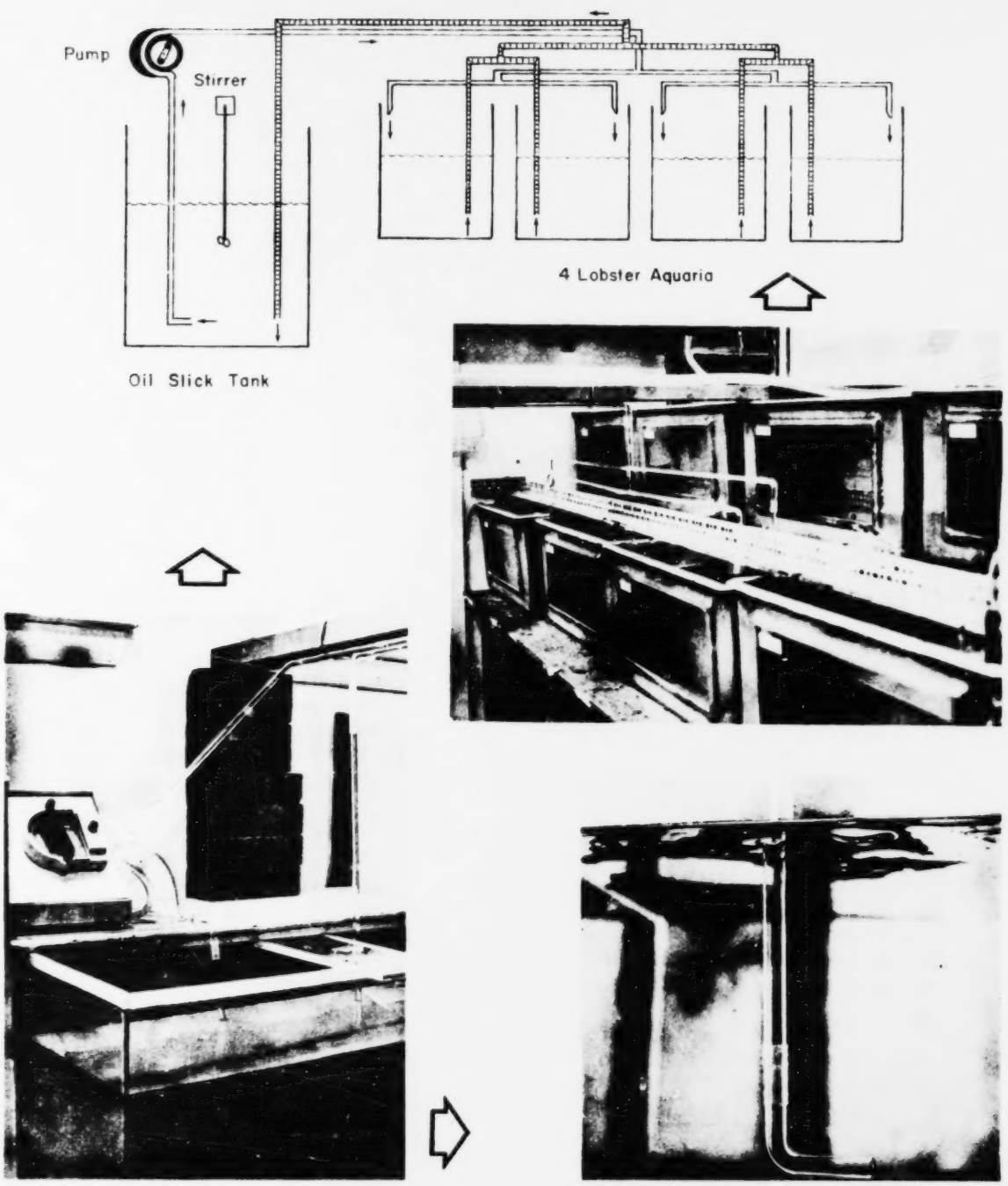


Figure 1. Connection of oil slick tank and lobster aquaria in solubles experiment.

was introduced on the water surface at 0900 hr. At 12 noon of that day (1) and on days two, three and five a volume of five liters was siphoned out of the center of the tank. Day four was not sampled to keep the tank volume sufficiently high for the lobster.

Each sample was extracted four times with 80 ml redistilled pentane. The resulting 320 ml pentane extract was evaporated down to about 2 ml for analysis by gas chromatography.

2. Gas Chromatography

Samples of the pentane extract (1/50 of volume) were injected in a column (8 ft. 1/16 inch, 0.2% Apiezon L on textured glass beads, 80-100 mesh), which was temperature programmed from 50-220°C at 8°/minute and maintained isothermally at 220°C. In order to identify the major peaks in the chromatograms a standard was run using 1 µl La Rosa crude dissolved in 50 µl pentane, of which 1/50 aliquots were used.

G. Determination of Hydrocarbons and Lipids in Seawater

A flow chart of the chemical analyses of lipid extract is presented in Figure 2.

1. Sampling and Extraction

Samples of lobster tank seawater before and after oil was introduced in the mixing tank, were taken each day at 12 noon starting at day five of the experiment. A volume of 1250 ml was siphoned out of the center of each test aquarium. The resulting volume (5 liters) was extracted four times with 80 ml redistilled pentane. The pentane extract was evaporated down to 2 ml for chemical analysis. Aliquots of this fraction were used for the following analyses.

2. Ultraviolet-Visible Spectra

Subsamples of the lipid extracts were dissolved in redistilled isooctane and spectra obtained using 1 cm quartz cells mounted in a Cary Model 14 spectrophotometer.

3. Infrared Spectra

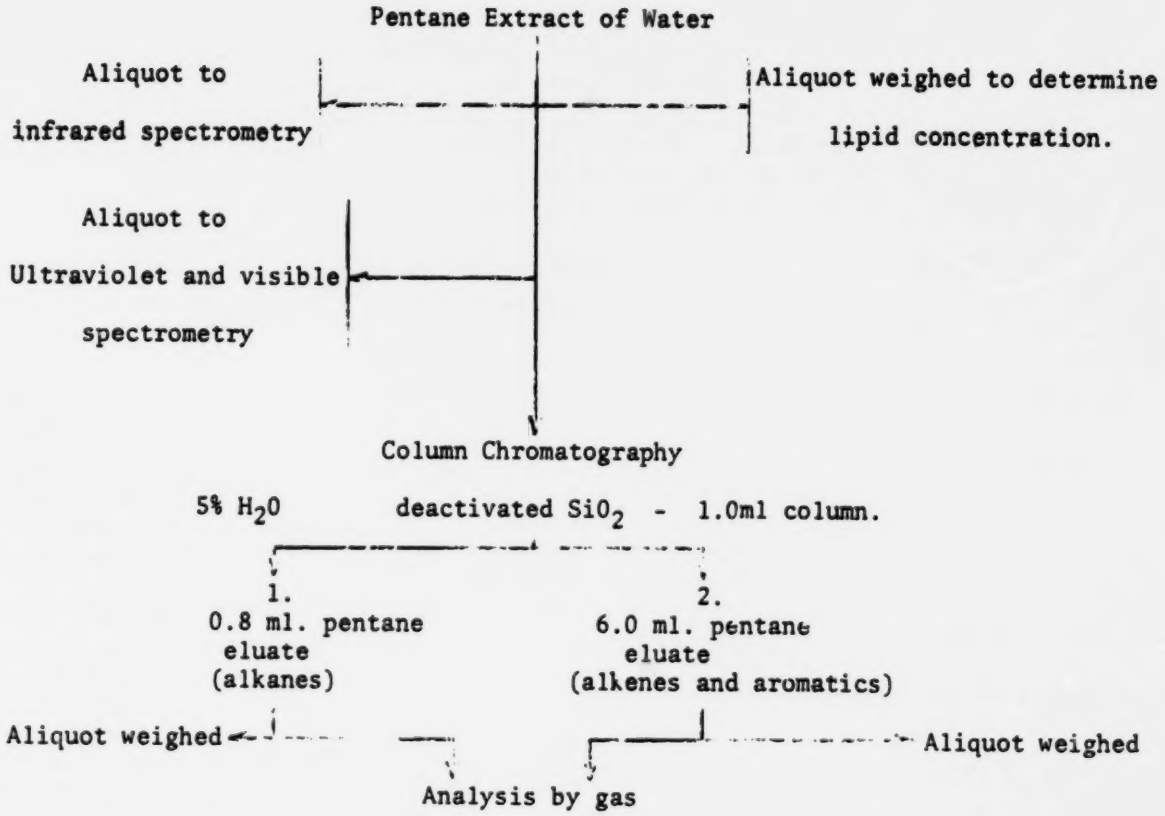
Subsamples of the lipid extracts were analyzed as thin films between a 1 x 4 mm NaCl crystal and a larger crystal mounted on a holder with a 1 x 4 mm mask. Spectra were obtained using a Perkin Elmer 337 infrared spectrometer with a beam condensor.

4. Column Chromatography (Methods of Blumer, 1970)

The lipid extracts (minus subsamples analyzed above) were dissolved in 0.2 ml of pentane and charged to a 1.0 ml column of silica. The silica had been deactivated with 5% water and extensively eluted with pentane prior to charging the sample to the column in 0.2 ml of pentane. The saturated hydrocarbons (alkanes) were eluted with 1.5 ml of pentane after collecting void volume. Alkenes and aromatic hydrocarbons were eluted with an additional 6.0 ml of pentane. The pentane was removed from each eluate fraction by evaporation under reduced pressure. The residual was taken up in 25-100 µl

FIGURE 2.

Flow Chart of Chemical Analyses



gas chromatography on 7.5 ft. 1/8" o.d.

3% Apiezon L /Chromasorb W 80/100 mesh.

temperature programmed 80-290^{0}C. at 6^{0}/min.

of CS_2 and 2.0-10.0 µl aliquots were weighed on a Cahn Electrobalance. The remainder of the sample was analyzed by gas chromatography.

5. Gas Chromatography

Hydrocarbons isolated from column chromatography were analyzed on a 7.5 ft. 1/8" 3% Apiezon L Chromasorb W 80/100 mesh column. The column was temperature programmed from 80-290°C at 6°/minute and maintained isothermally at 290°C. All solvents employed in this analysis were redistilled. Analysis of a blank showed no contribution by solvents and other chemicals to the reported concentrations of lipids and hydrocarbons. UV absorbance values were corrected for absorbance contributions from the solvents and chemicals.

H. Microscopy of Chemosensory Hairs

In order to reveal possible structural damage of exposure to oil the chemosensory hairs on the antennules, the so-called aesthetasc hairs were examined by light and electron microscopy. Immediately after completion of the experiments both outer rami of the antennules of the experimental animals were cut. One ramus of each animal was used for light microscopy, the other one for electron microscopy.

1. Light Microscopy

Freshly cut outer rami with aesthetasc hairs were kept in seawater and immediately viewed and photographed with high power light microscopy and oil immersion at 1000 x magnification. A Wild microscope was used with plan focal objectives and high power electron flash to reduce exposure time and improve contrast. To further decrease exposure times (to 1 m sec.) pictures were taken on Kodak Tri X film exposed and developed at 1600 ASA in Acufine developer. This procedure resulted in satisfactory pictures with good contrast, and relatively free of vibration "fuzz".

2. Electron Microscopy

Antennular outer rami were cut and fixed immediately in 6% glutaraldehyde in seawater for four hours. Further fixation in 2% phosphate buffered osmium tetroxide was done for 16 hours, after which the specimens were dehydrated in an ethanol series. At 70% ethanol, the aesthetasc hairs were carefully removed from the antennule under a dissecting microscope. In some cases individual segments were cut off the ramus leaving the two rows of hairs attached to each segment. Dehydration was then completed, after which the hairs were embedded in araldite going through the following mixutres of araldite and propylene oxide: 0:1 - 0:1 - 1:3 - 1:1 - 1:0. The plastic was polymerized during 72 hours of baking at 70°C. Control specimens were prepared from fresh lobsters using identical procedures. All specimens were cut and viewed in an Hitachi electron microscope.

SECTION V

RESULTS

A. Analysis of Behavior

The behavior units listed in Table I were analyzed individually for changes in their frequency of occurrence during the first five days versus the last five days. A sample treatment was already given for the unit "Crude, Antenna Wave". All units were treated in this fashion. The complete set of results can be found in Appendix Table II.

Three behavior units were found to change significantly: A x^2 test showed significance at the 0.01 level for "Crude, Antenna Wave", Crude, Antennule Wave" and "Crude, Fan Slow". All three units increased in frequency in the last five day period compared with the first five day period. The frequency change of the unit "Crude, Beat Fast" 0.05 level, but here a lower frequency of occurrence appeared in the last period.

These units were only significant in the Crude Oil experiment, but not in tests with the Soluble fraction. In these experiments, none of the behavior units changed significantly.

B. Analysis of Feeding Times

The three time phases of feeding behavior were analyzed for changed in duration in the first five days versus the last five days. Treatment was identical to that of the frequency of behavior units. The time in seconds is listed in Appendix Table III for the complete set of data.

A significant change (0.01 significance) in feeding time between the first and the last five days was found only for the phase "Crude, Wait". The average "Crude, Wait" time was about doubled in the last five days and represent the greatest change in the lobsters' behavior. The mean wait time in the first five days was 17.2 sec. versus 36.4 sec., in the last five days. No other significant changes occurred.

C. Oil Slick Photographs

A visual idea of the oil slick at days 6-10 can be formed from the photographs that were taken each day (Figures 3 and 4). The coherent slick of day six is broken up at day seven and finely dispersed at day eight. In the first series a fine granular appearance of the slick is evident on days nine and ten. In the second series the slick is probably mixed in with the water to some extent after day nine due to malfunctioning of the stirrer. Mixing was obvious at day ten, when practically no oil was visible at the water surface.

First series, oil in female tanks, March 10-14, 1972

1a. One hour after oil introduction (4.5 ml La Rosa crude); oil covers about 20% of the water surface in an almost solid black-brown slick (Figure 3A).

1b. Seven hours; oil covers the entire surface, broken up in vacuoles up to 4 cm in diameter slightly lighter color (Figure 3B).

2. Twenty-five hours; oil globules up to 1 cm in diameter cover entire surface (Figure 3C).

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3. Forty-eight hours; same as twenty-five hours (Figure 3D); more stirrer action visible.

4. Seventy-three hours; some thin 1 cm globules still present, but appearance has become granular and gray (Figure 3E); much oil has disappeared from surface.

5. One hundred hours; as seventy-three hours (Figure 3F); some oil has stuck to the walls (Figure 3G).

Second series, oil in male tanks, March 22-26, 1972

1. Ten minutes after oil introduction (4.5 ml La Rosa, crude); solid black slick covers about 10% of water surface (Figure 4A).

2. Twenty-seven hours; an oil film with large (up to 3 cm diameter) and small vacuoles covers about 85% of surface (Figure 4B).

3. Fifty-five hours; a thin film full of small vacuoles (1 cm diameter) covers the entire surface (Figure 4C).

4. Seventy-four hours; very thin granulated cover of oil over whole surface (Figure 4D); some is coating walls. Stirrer malfunction suspected.

5. Ninety-seven hours; almost all oil has disappeared (Figure 4E) some is coating walls. Stirrer malfunction evident, though not directly observed.

D. Analysis of Hydrocarbons and Lipids in Oil-Sea Water Mixture

Gas chromatograms for pentane extracts of 1) La Rosa crude oil and 2) oil-water mixtures at day six; 3) day seven; 4) day eight; and 5) day ten are shown in Figure 5. Major peaks can be matched well and their fate followed over five days. The greatest observable effect is the decrease of low boiling compounds and the increase in the unresolved envelope. In general individual peaks disappear in favor of the unresolved fraction; see also below.

E. Analysis of Hydrocarbons and Lipids in Sea Water

1. Hydrocarbon Concentrations and Lipid Concentrations

The results of the analyses of lipid concentrations and hydrocarbon concentrations are presented in Table III. The important aspects of the data are as follows:

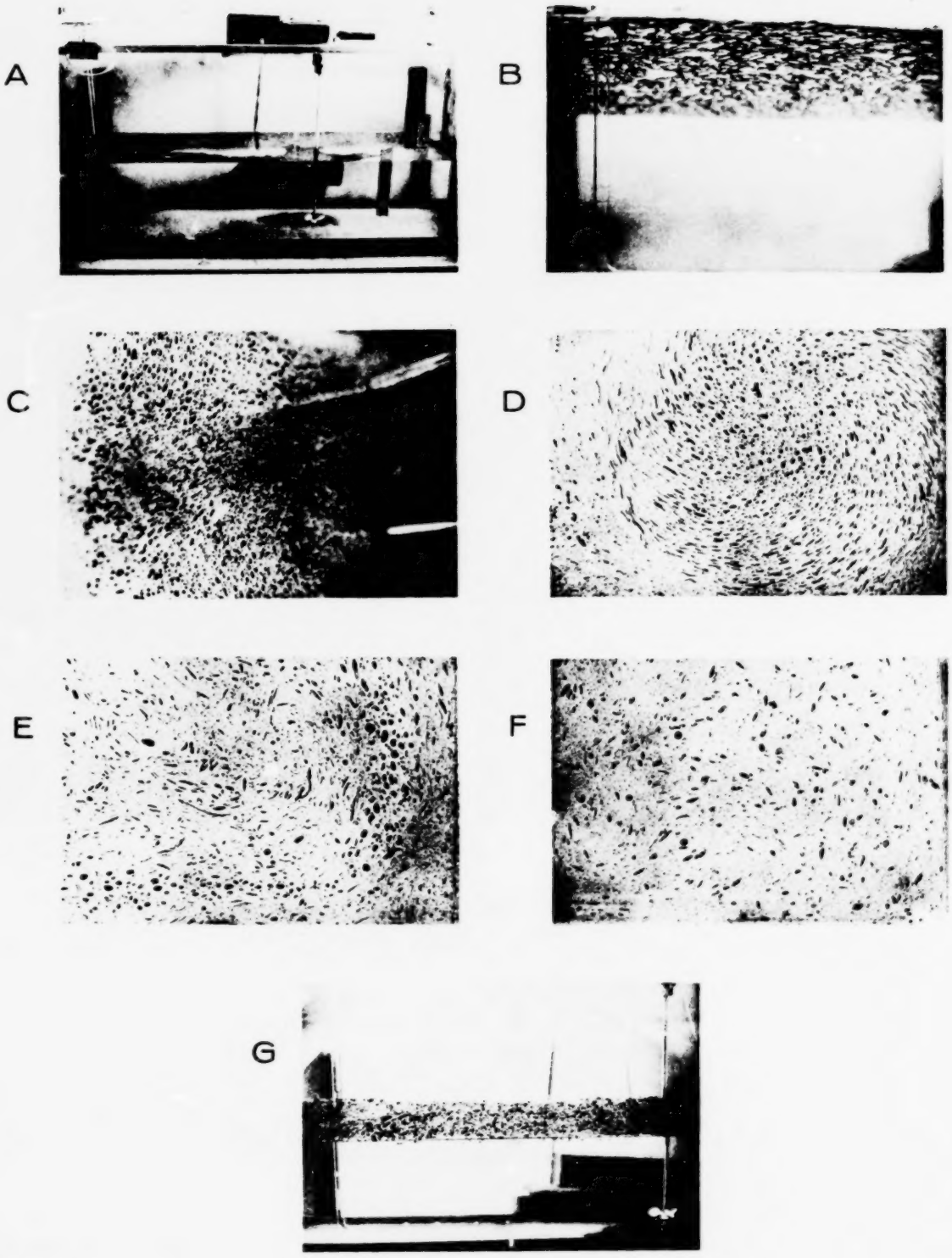


Figure 3. Appearance of oil slick over five day period, first series of solubles experiment.

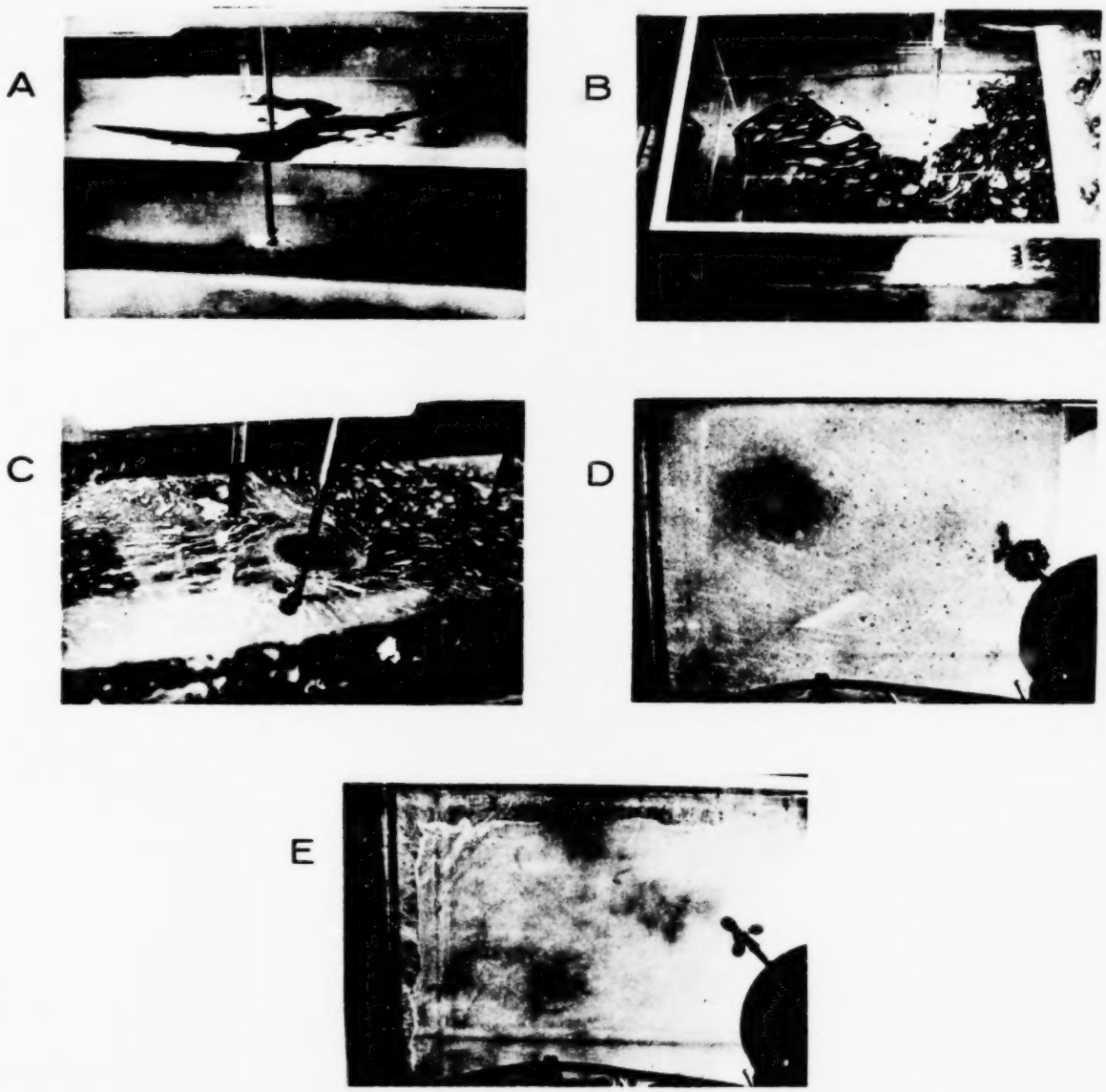


Figure 4. Appearance of oil slick over five day period, second series of solubles experiment.

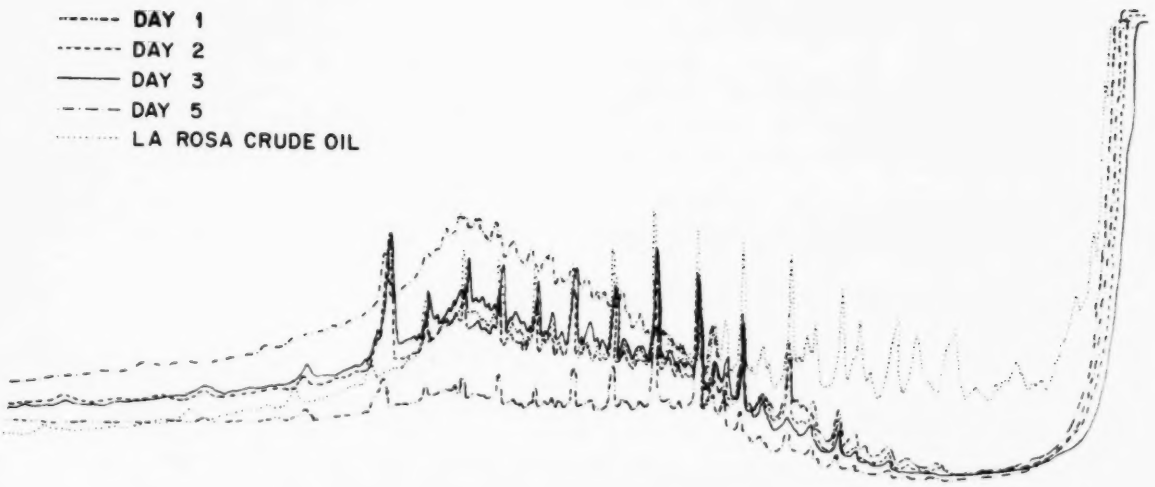


Figure 5. Gas chromatograms of pentane extract from oil-water mixture.

a) Lipid concentration decreases markedly following connection of the oil mixing tank into the water circulating system of the experimental tanks and continues to decrease until day nine where the trend is reversed. The day ten lipid concentration is high as a result of the mixing of the oil slick throughout the oil-mixing tank and into the experimental tanks when the stirring motor malfunctioned.

b) Total hydrocarbon concentration decreases after the oil-slick tank is connected into the water circulating system. The total hydrocarbon concentration then fluctuates until day nine where the concentration decreases and then increases again on day ten due to the stirrer malfunction.

c) The alkane concentration essentially parallels that of the total hydrocarbon concentration.

d) The total alkene + aromatic hydrocarbon fraction concentration fluctuates at a value near the lower detection limit of the methods employed until day nine where an increase is noted. On day ten the concentration again increases as expected when the stirrer malfunctioned.

e) Generally the ratios of hydrocarbons to lipids, alkanes to lipids, and alkenes + aromatics to lipids all increased throughout the course of the experiment with the exception of a slight decrease from day seven to day eight. The ratio of the alkenes + aromatics to lipids shows the greatest increase of the three ratios.

f) The ratios of alkanes/alkenes + aromatics shows a definite decrease after day six and fluctuates at lower values through day ten.

2. Ultraviolet-Visible Spectra

Comparison of the UV spectra given in Figure 8 with appropriate reference to the dilution factors of the lipid extract showed a steady increase in the UV absorbance during the experiment after the connection of the oil slick tank. Four absorbance bands were clearly defined in the spectra and the absorbance of each band calculated per unit volume of sea-water and corrected for absorbance contributions of the solvents and chemicals used in isolating the lipids from the water. Corrections were less than 0.005 absorbance units and were small compared to the absorbance measured for the samples except for day five where the absorbance of the sample was due entirely to the absorbance of unknown compounds contributed by the solvents and chemicals of the extraction procedure. The absorbance values of the four selected bands are given in Table IV.

UV spectra of the alkene-aromatic hydrocarbons isolated by column chromatography from the day ten lipid extract and the UV spectra of aromatic hydrocarbons isolated from the La Rosa crude oil used in the experiment are presented in Figure 7. The spectra are essentially the same. A comparison of the UV spectra of the alkene-aromatic hydrocarbon fraction of the lipid extract of day ten shows a substantial difference. This difference could be due to UV absorption of polynuclear aromatic hydrocarbons present in the lipid extract, but not eluted from the column or the presence of non-hydrocarbon compounds soluble in pentane and absorbing in the UV region.

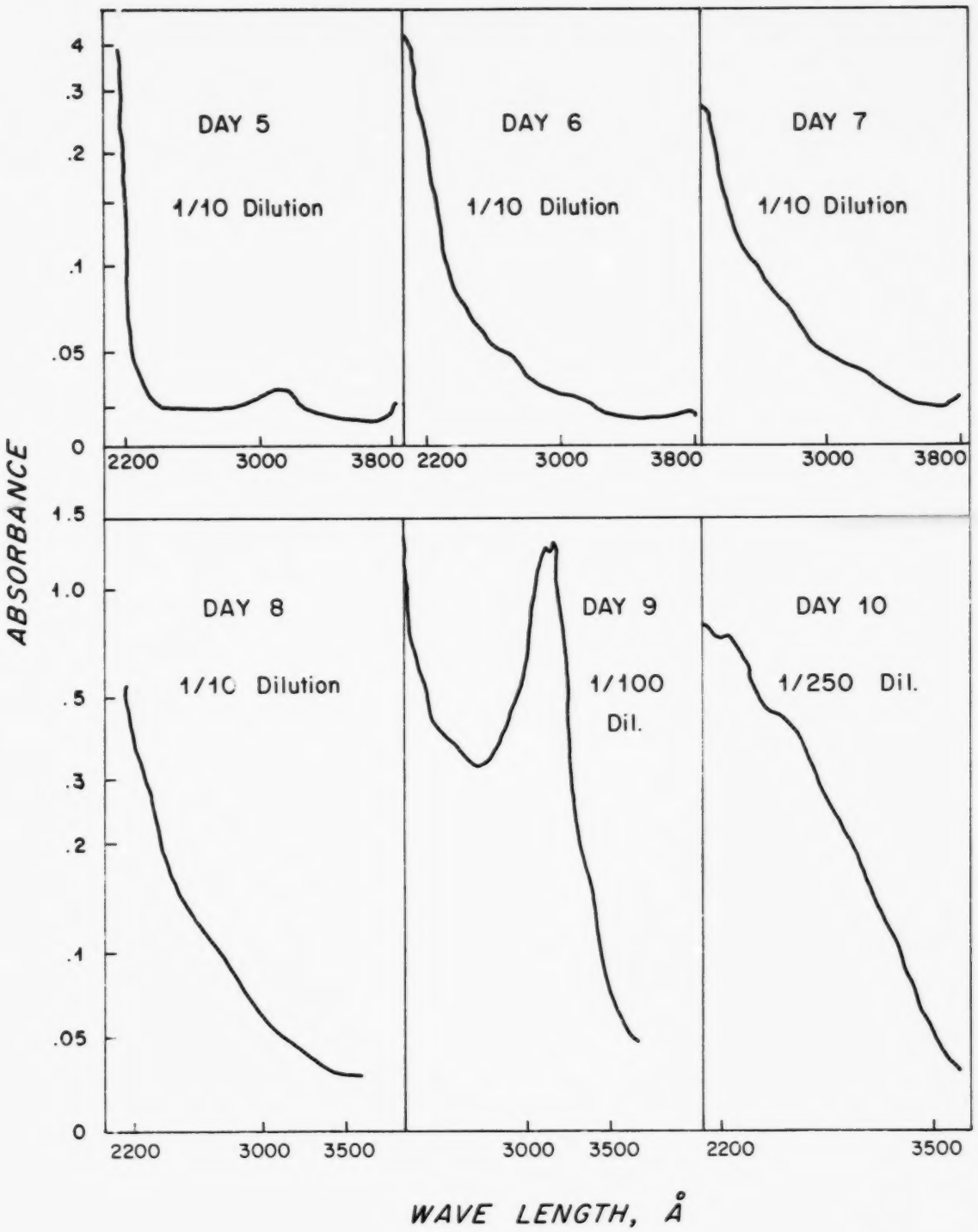


Figure 6. Ultraviolet spectra of lipid extracts of solubles on days 5-10.

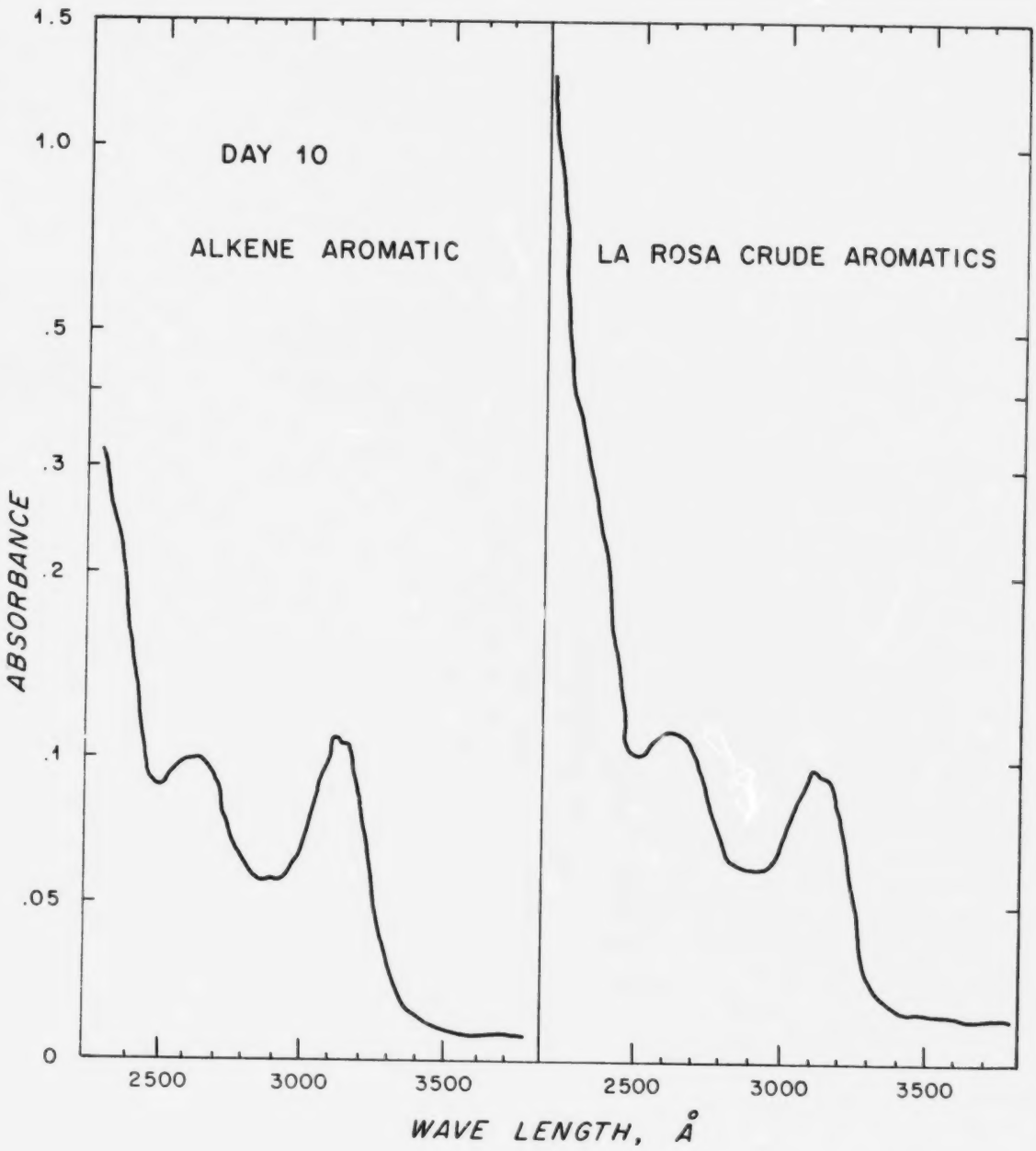


Figure 7. Ultraviolet spectra of alkene-aromatic fraction of day 10 and of whole crude oil.

TABLE III - LIPID CONCENTRATIONS AND

HYDROCARBON CONCENTRATIONS IN SEAWATER

	Day					
	5	6	7	8	9	10
Lipids (µg/liter)	1076	232	182	109	370	1786
Total Hydrocarbons (µg/liter)	43.0	17.2	24.5	12.4	91.8	541.0
Alkanes (µg/liter)	37.8	15.1	16.3	8.9	59.7	290.0
Alkene-Aromatic Hydrocarbons (µg/liter)	5.16	<2.01*	8.29	3.51	32.1	251.0
RATIOS						
Total Hyd/lipid	0.040	0.074	0.135	0.114	0.248	0.303
Alkanes/lipid	0.035	0.065	0.090	0.082	0.161	0.162
Alkene-Arom/lipid	0.005	0.009	0.046	0.032	0.087	0.140
Alkane/alkene-arom.	7.32	7.23	1.97	2.54	1.86	1.16

* Concentration could not be more than 2.01, the lower detection limit and could be much less

TABLE IV - ULTRAVIOLET ABSORBANCE OF LIPID EXTRACT OF ONE LITER OF SEAWATER

Wavelength Å	Day 5	6	7	8	9	10
2280	--	0.604	0.514	0.676	18.20	14.60
2580	--	0.158	0.194	0.232	4.30	8.84
2750	--	0.104	0.125	0.166	3.66	6.16
3120	--	--	0.017	0.008	16.30	2.14

There were no detectable absorbance bands in the visible wavelength region except for days nine and ten where weak absorbance was noted at 4000 Å.

3. Infrared Spectra

The absorbance values for the absorption bands noted in the IR spectra of the lipid extracts are given in Table V. The values are absorbances normalized to the absorbance at 2955 cm^{-1}. There has been insufficient time to fully analyze the data. However, it is clear that the composition of the lipids in the water is changing as is evidenced by a decrease in absorbance at some wavelengths and increases in absorbance at other wavelengths.

4. Gas Chromatography

The gas chromatograms of alkanes for days 5, 6, 7 and 8 (Figure 8) showed very similar patterns with resolved peaks assigned to a series of n-alkanes and branched alkanes eluting over a signal due to an unresolved complex mixture of hydrocarbons. The signal due to the unresolved complex mixture increased markedly on day nine and increased again on day ten (Figure 8). Comparison of the gas chromatogram of alkanes present on day ten with the alkanes isolated from the La Rosa crude oil used to form the oil slick showed a depletion of the lower boiling hydrocarbons and a depletion of the n-alkanes and branched alkanes relative to the unresolved complex mixture. This unresolved complex mixture contains many hundreds of cyclic and branched cyclic hydrocarbons - usually designated by the term naphthenes and are characteristic of gas chromatograms of alkanes isolated from crude oils and fuel oils.

The gas chromatograms of the alkene-aromatic fractions (Figure 9) of the hydrocarbons showed the same features on days 5, 6, 7 and 8 with only three peaks at retention indices 2044, 2081 and 2107 on Apiezon L present in the gas chromatograms. No detectable unresolved complex mixture was present. On day nine and day ten there was an unresolved complex mixture signal present in the gas chromatograms. The three peaks were also present eluting over the complex mixture signal. The unresolved complex mixture on the day nine and day ten chromatogram was probably due to naphtheno-aromatic hydrocarbons.

F. Microscopy

A morphological description of the olfactory sense hairs of the lobster, the aesthetasc hairs, is given in Figure 10. These hairs are primary candidates for disruption by chemical pollutants because their internal structures, the olfactory dendrites and cilia, are probably immediately accessible to rather large molecules in the environment (Ghiradella, 1968). The other non-chitinous body covering is found in the gill membranes, which were not investigated. The rest of the lobster is covered with hard chitin, impregnated with calcium.

No consistent differences other than normal variations could be observed either at the light microscopical external level in vivo or at the electron microscopical internal level after EM preparation (Figures 11-18).

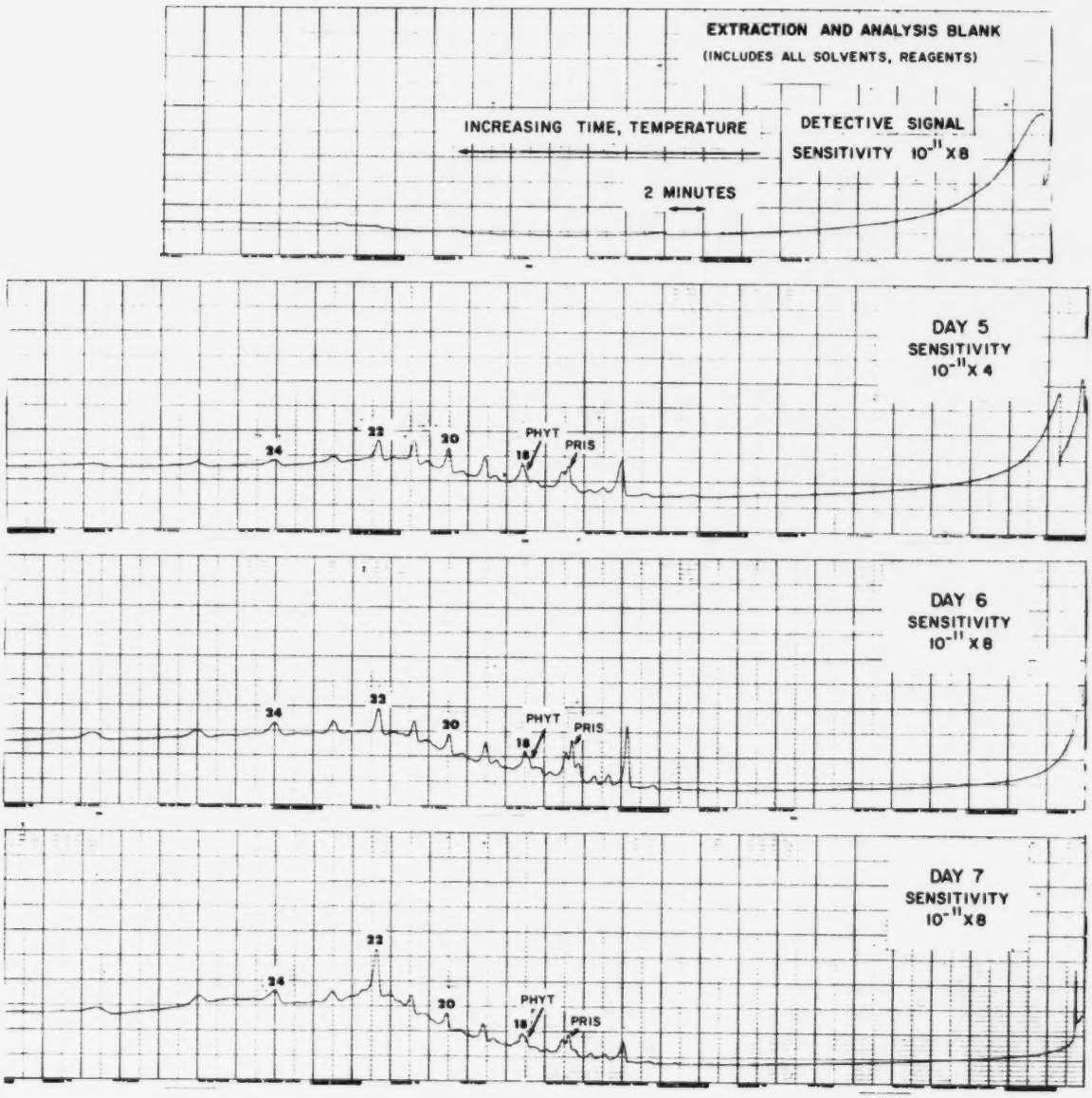
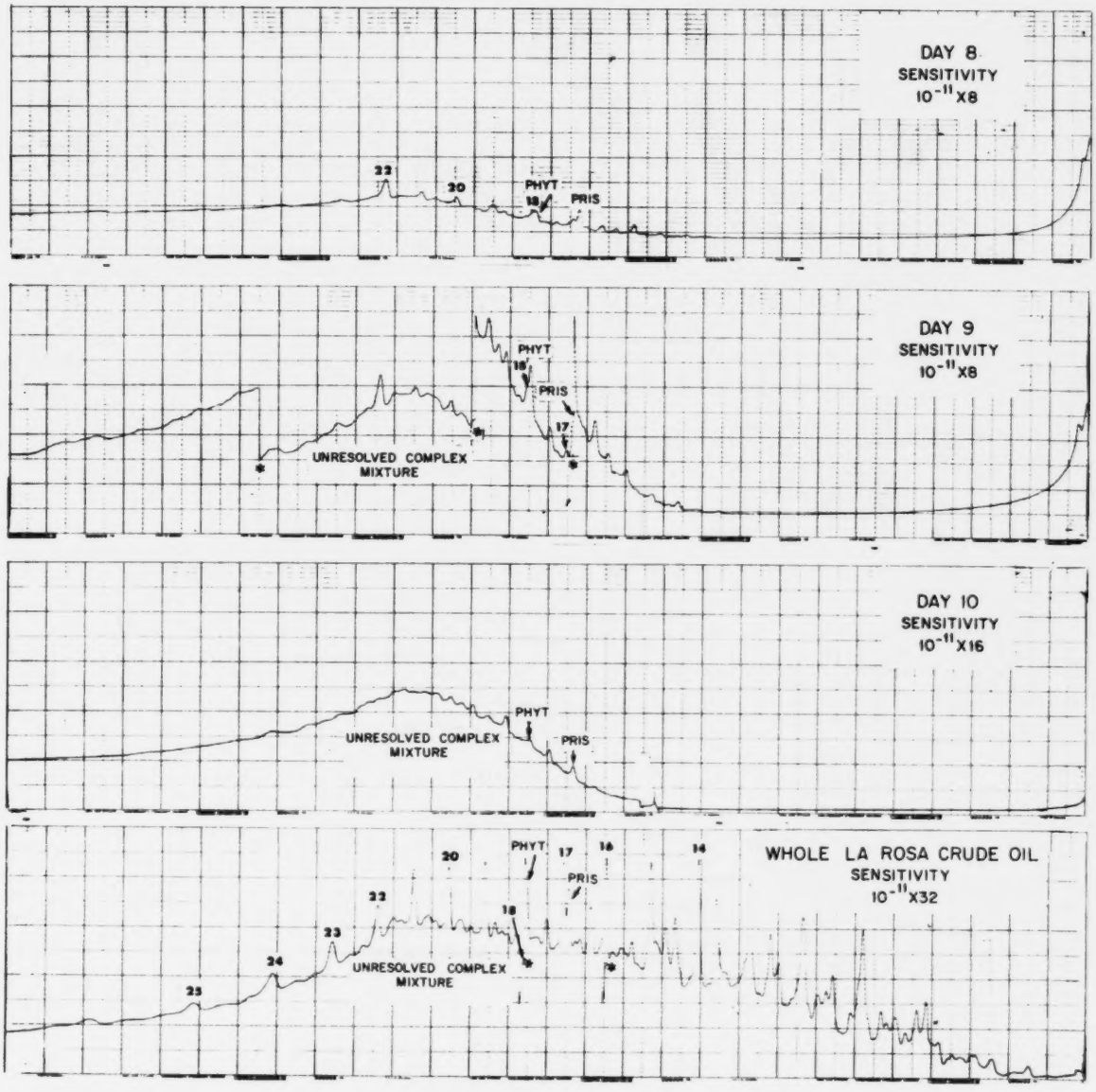
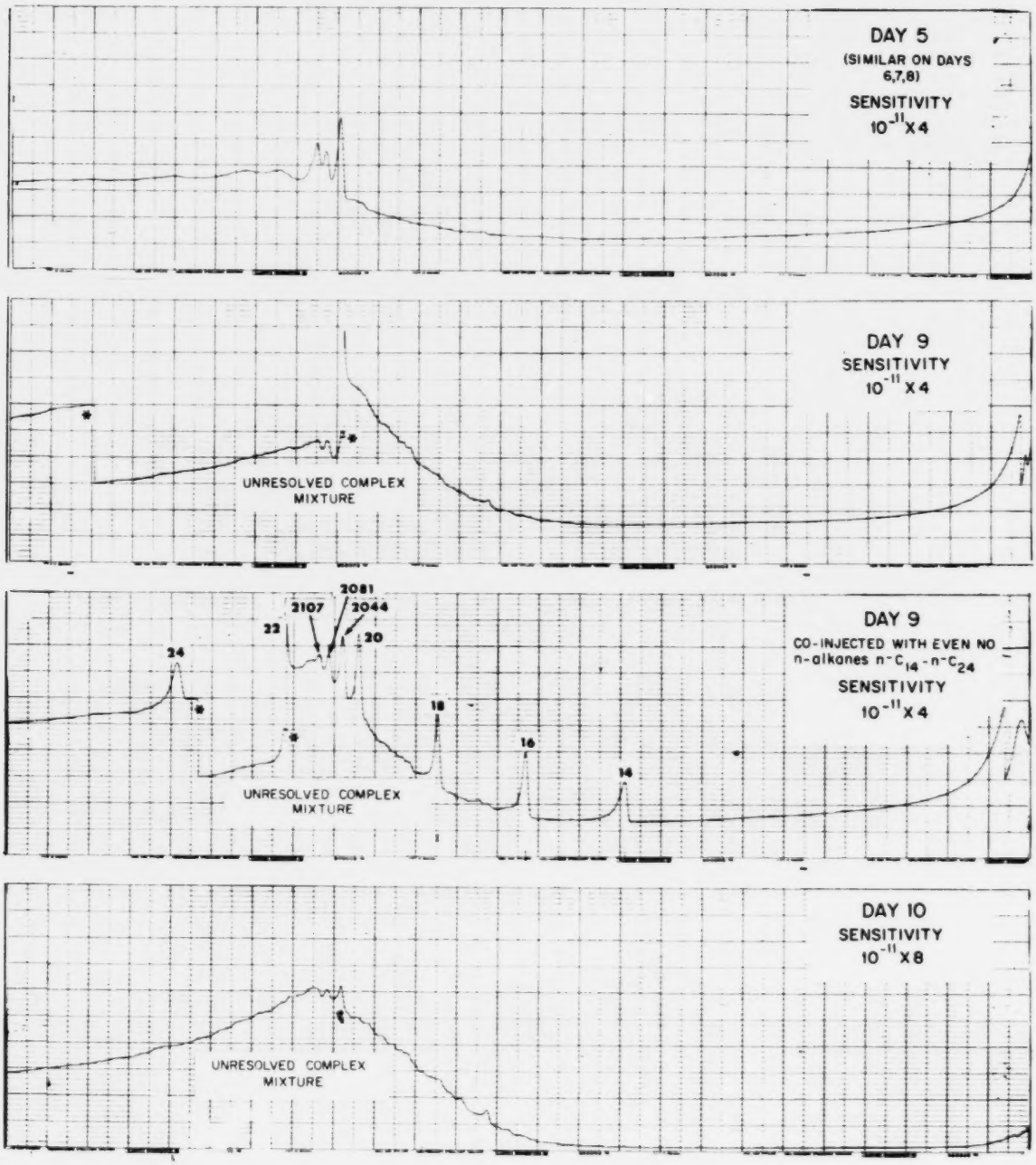


Figure 8. Gas chromatograms of alkane fraction of solubles.



* Automatic attentuation of detector signal.
Pris- Pristane, Phyt- Phytane. Numbers refer to carbon chain lengths of n-alkanes. Identifications are tentative only and are based on coinjection with even numbered n-alkanes and/or interpolation between even-numbered n-alkanes to obtain the retention index g., 2044 peak.

Figure 8. Gas chromatograms of alkane fraction of solubles.



* Automatic attentuation of detector signal.
Pris- Pristane, Phyt- Phytane. Numbers refer to carbon chain lengths of n-alkanes. Identifications are tentative only and are based on coinjection with even numbered n-alkanes and/or interpolation between even-numbered n-alkanes to obtain the retention index e.g., 2044 peak.

Figure 9. Gas chromatograms of alkene-aromatic fraction of solubles.

TABLE V - INFRARED ABSORBANCES NORMALIZED TO 2955 Cm^{-1} ABSORBANCE BAND

	Day					
cm^{-1}	5	6	7	8	9	10
3000	--	.10	.23	.16	.10	0.05
2955	1.000	--	--	--	--	--
2915	.19	1.40	1.03	1.55	1.69	1.59
2850	.10	.75	.80	.74	.77	.73
1735	.05	.45	.53	.58	.13	.05
1715	.04	.35	.43	.37	--	--
1626	.02	--	.03	--	--	.07
1450	.06	.30	.42	.32	.31	.30
1410	.10	--	--	--	--	--
1379	--[a]	.30	.12	.18	.46	.16
1265	4.69	1.10	.35	.45	.46	.07
1100	4.31	.95	.22	.34	.59	.07
1029	4.43	1.10	.27	.45	.59	.09
870	0.81	0.35	.10	.08	--	--
810	5.75	1.15	.15	.37	.59	.07
760	0.81	0.50	0.13	.18	.46	.11

a -- not detected.

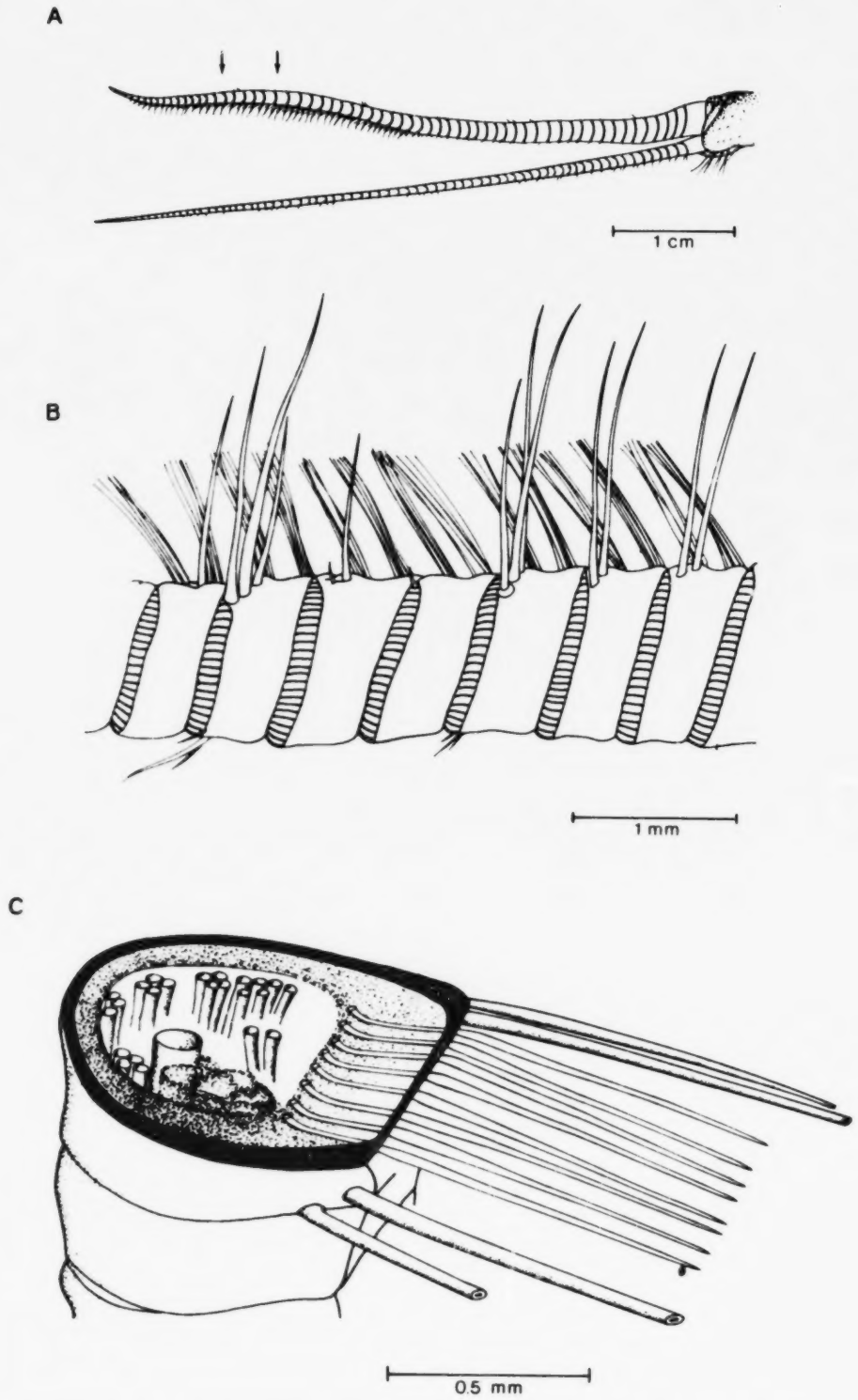


Figure 10. Antennule with aesthetasc hairs and guard hairs.

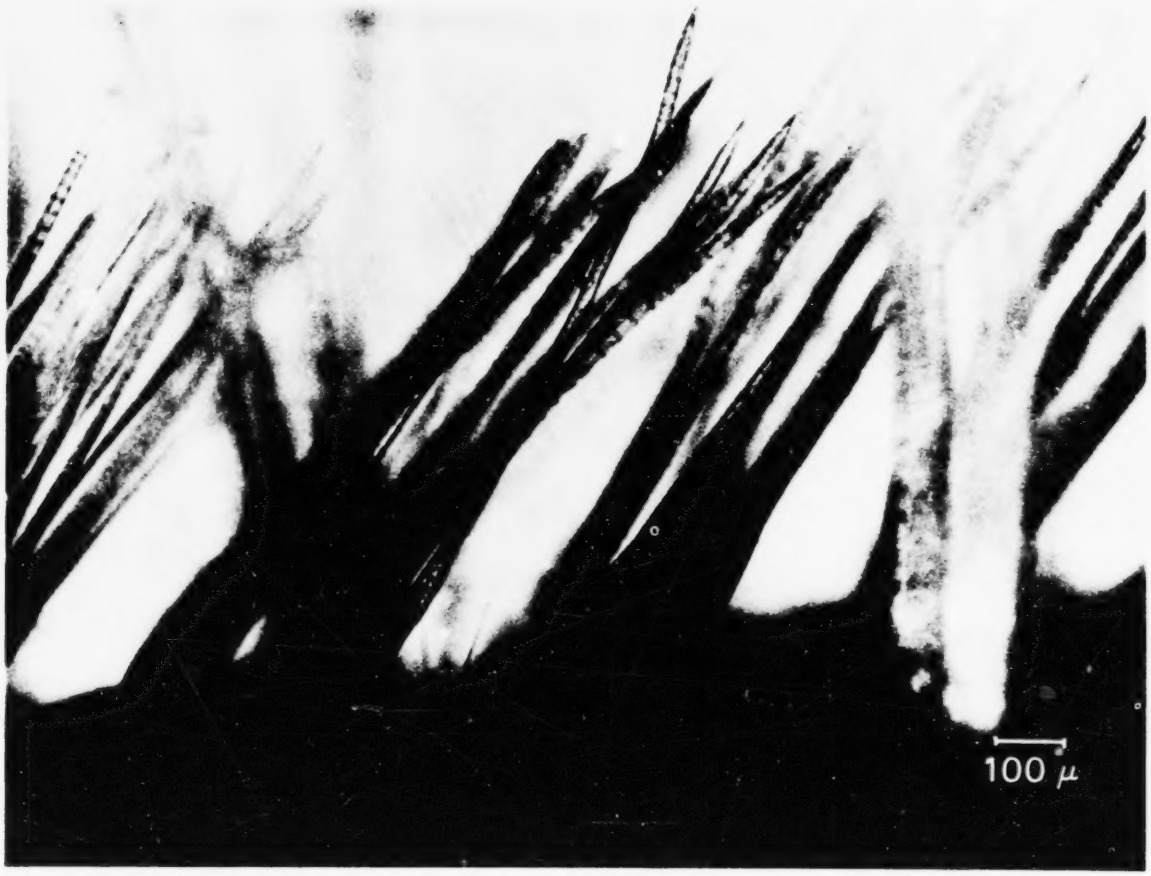


Figure 11. Antennule in vivo (150x, light microscopy).

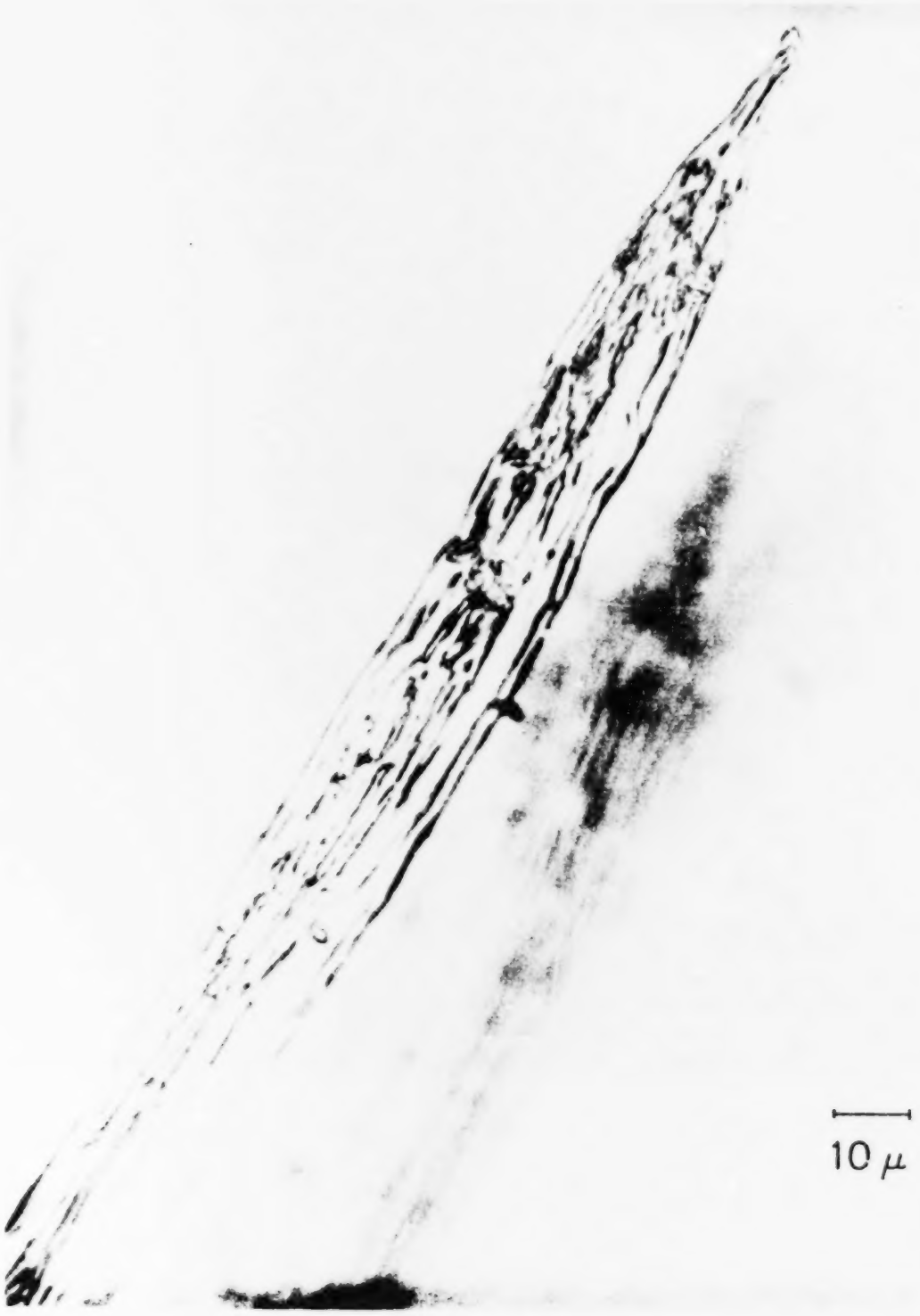


Figure 12. Detail of tip of aesthetasc hair vivo (1500x, light microscopy).

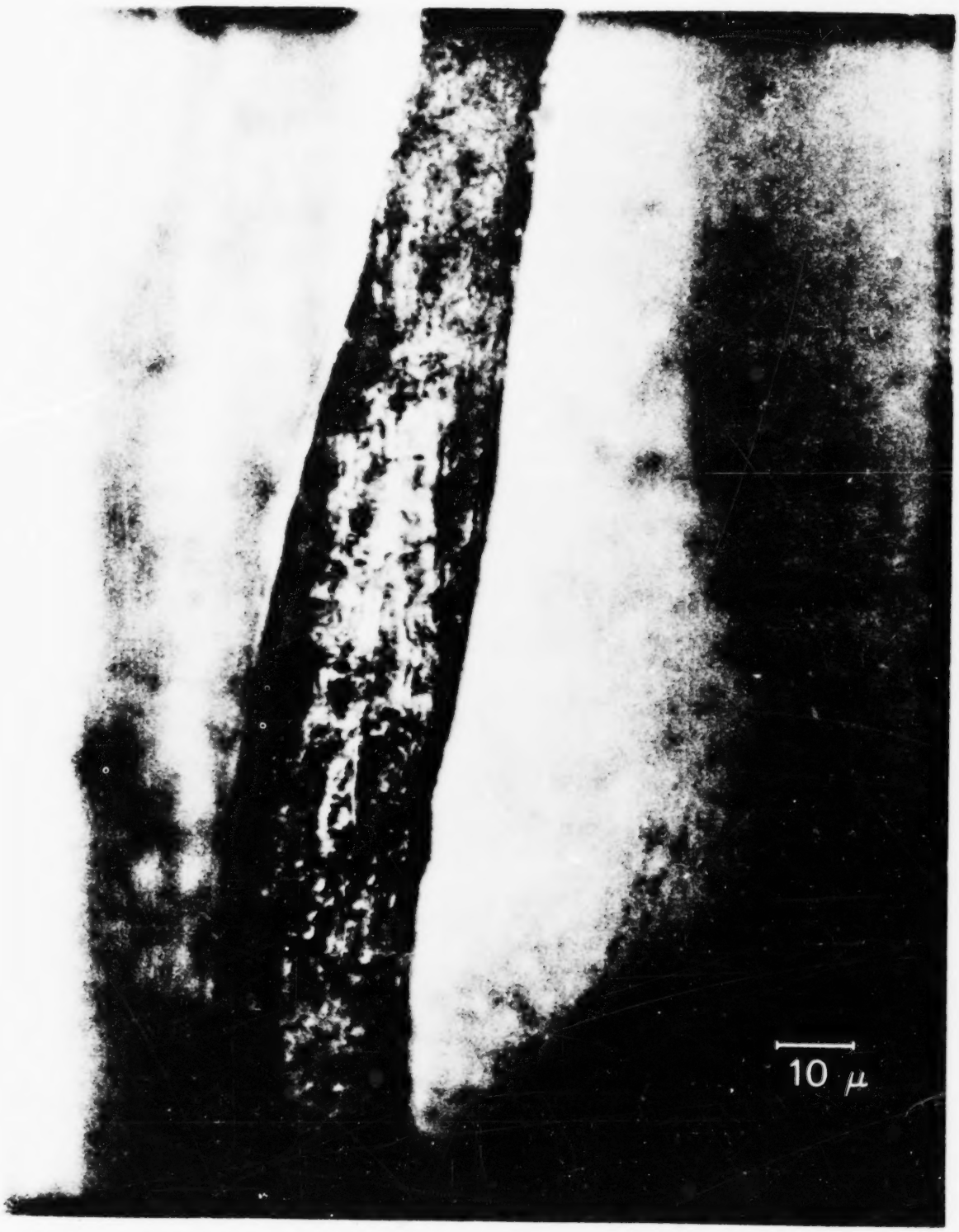


Figure 13. Detail of base of aesthetasc hair in vivo (1500x light microscopy).

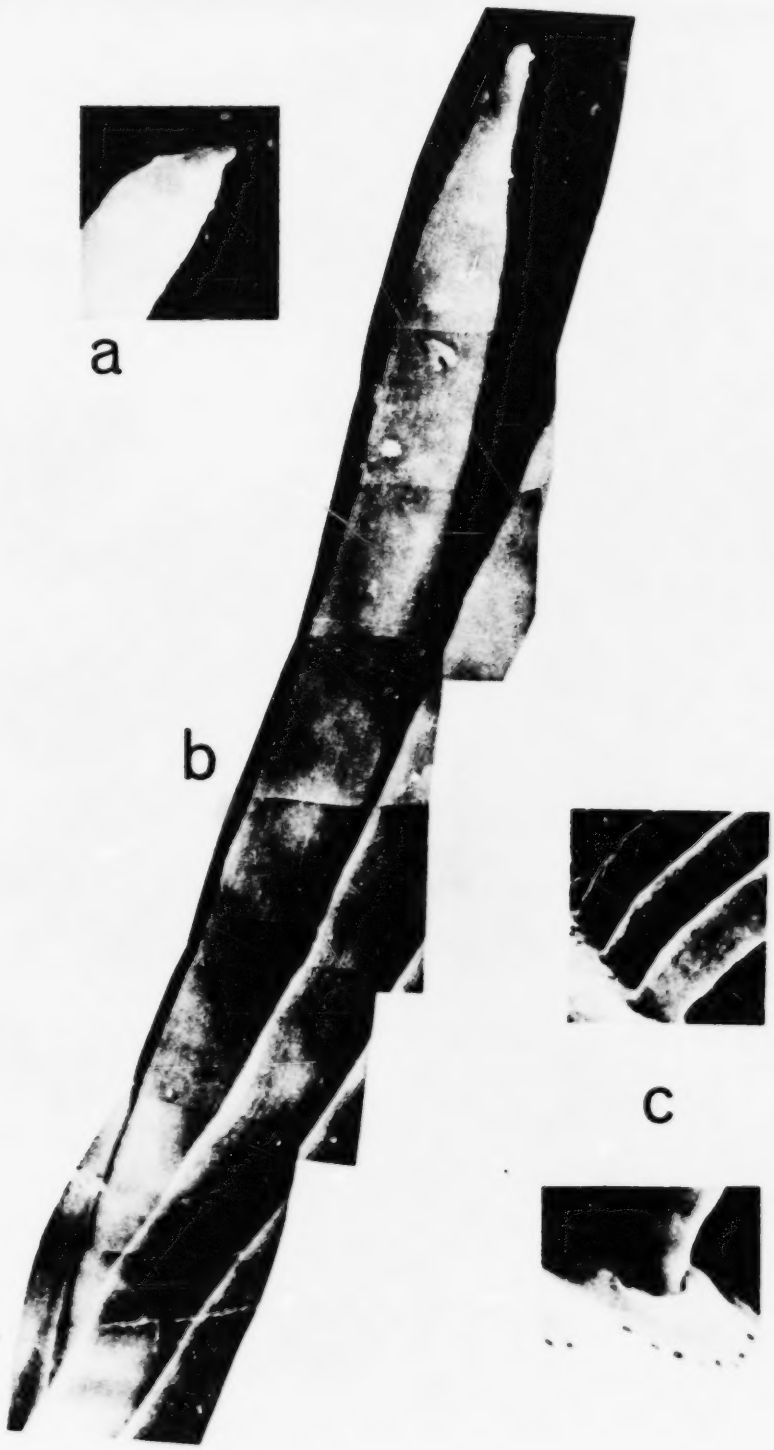


Figure 14. Aesthetasc hair details (scanning electron microscopy).
a) Tip, head-on view
b) distal 1/2 portion
c) base, head-on view, with pores in antennule

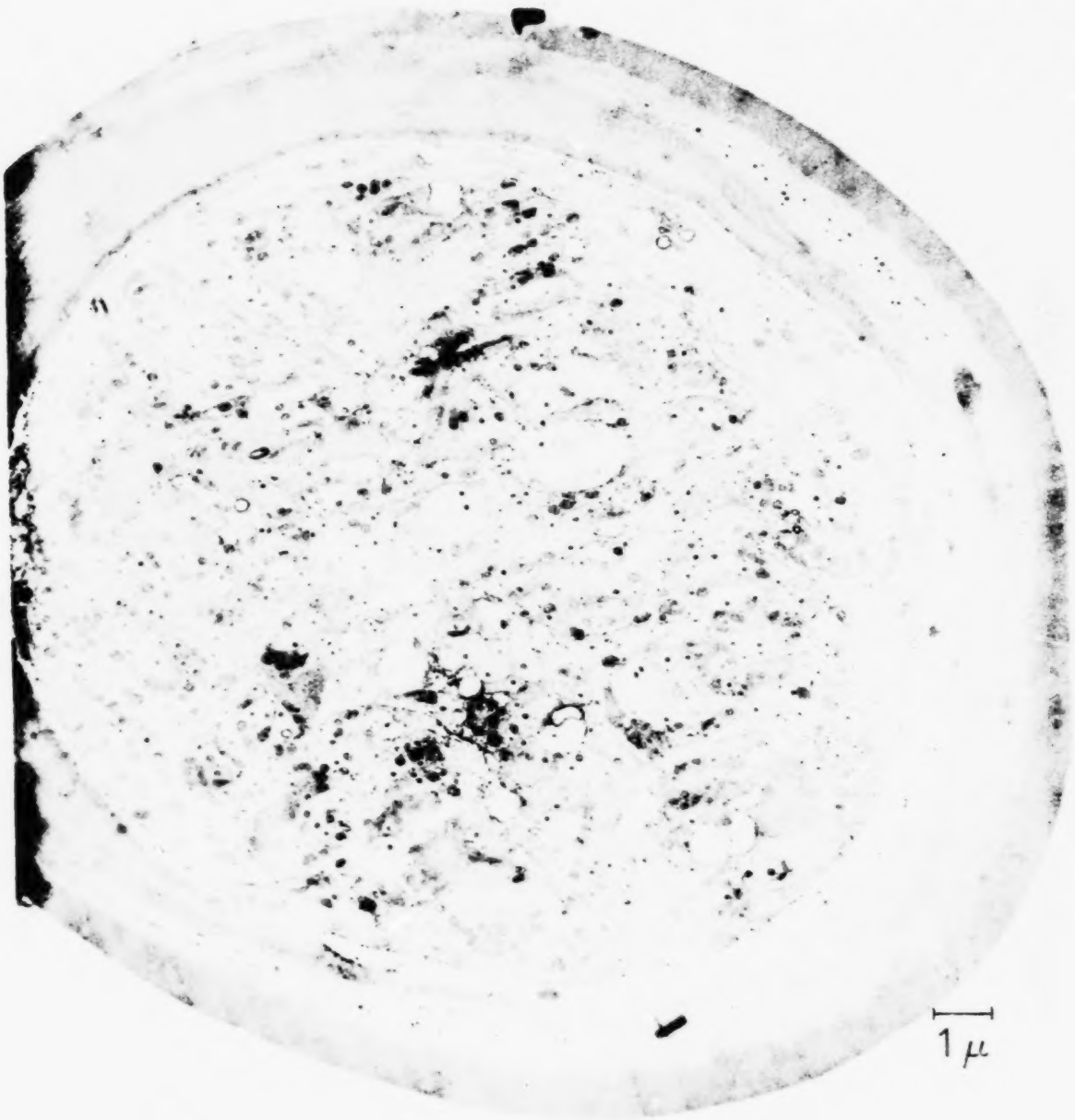


Figure 15. Cross section of middle portion of aesthetasc hair (12,000x electron microscopy).

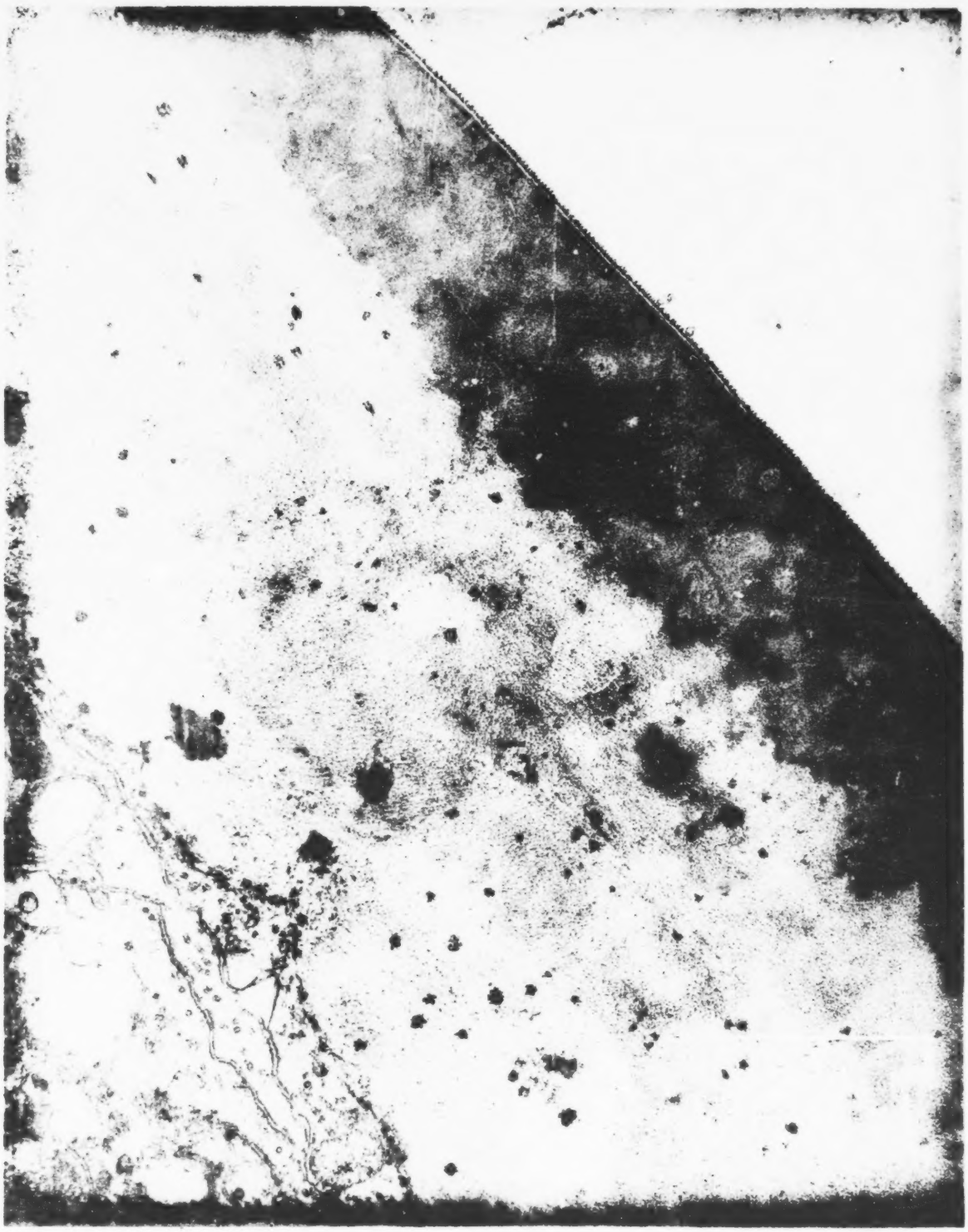


Figure 16. Detail of cuticle of Figure 15. (45,000x electron microscopy).

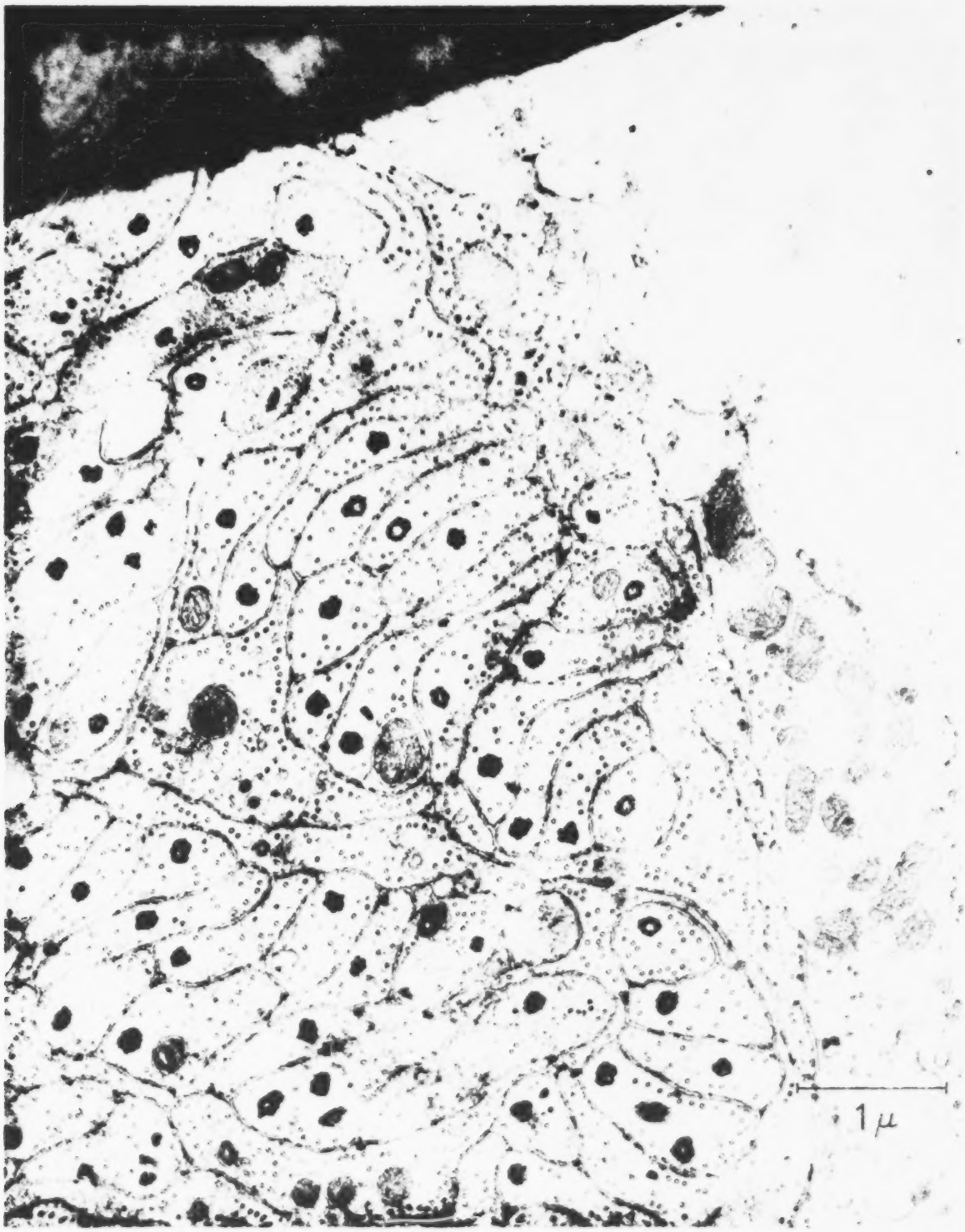


Figure 17. Detail of dentrites inside aesthetasc hair middle portion (33,000x electron microscopy).

Figure 18. Detail of dendrites inside aesthetasc hair middle portion (29,000x electron microscopy).

SECTION VI

DISCUSSION

A. Fate of Oil in Seawater

Some of the results from the more extensive chemical analyses performed on the water soluble fraction can be expected to be generally valid for the crude oil-water mixture also. Water analysis on day five before oil exposure should be similar in both cases. It may be expected that, since oil emulsified in the first experiment, bacterial degradation takes place faster. A comparison of the gas chromatography results of both experiments shows, indeed, great similarities in overall similarities with time.

The results of hydrocarbon analyses show that there were probably some petroleum hydrocarbons present in the experimental tanks prior to exposure to an oil-slick. This could have been the result of contamination of the natural seawater in the experimental tanks, or despite extensive cleaning and leaching procedures, due to contamination of the tanks with oil from a previous experiment.

The decrease in lipid concentration from day five to day six and continuing through day eight could be due to adsorption of lipids onto the walls of the oil-slick tank or into the oil slick itself. The addition of water to the system would only dilute the lipid concentration to a value of 800 µg/liter, well above that of the 232 µg/liter measured on day six.

The data of Tables III, IV and V and Figures 6 and 7 all indicate that the lipid composition, lipid concentration and hydrocarbon concentration are changing throughout the experiment. The exact processes involved are not clear. Chromatograms in Figures 5 and 8 suggest that bacteria and other microorganisms are degrading the oil.

The comparison of the gas chromatograms of alkanes of La Rosa crude and day ten alkanes show that the oil present in the tanks after five days has been biochemically oxidized to the extent that n-alkanes and branched alkanes have been reduced in concentration relative to the naphthenes (unresolved complex mixture). This is expected in view of the preferential oxidation of n-alkanes and branched alkanes relative to the naphthenes as demonstrated by the many investigations reviewed by ZoBell (1969).

The comparison of the UV spectra of lipids extracted on day ten and the UV spectra of the alkene-aromatic fraction of hydrocarbons isolated from the day ten lipid extract suggests that some of the UV absorption of the day ten lipid extract is due to other than aromatic hydrocarbons or to higher molecular weight aromatics than those eluted from the column by the procedures employed -- i.e. more complex polynuclear aromatics than phenanthrene and anthracene.

The increase in the hydrocarbon concentrations on day nine may have been due to a short term malfunction of the stirrer which was not noted by visual observations. This is conceivable in view of the failure of the stirrer on day ten (also not observed).

In conclusion, the data indicate that several processes may be operating at the same time during the course of the experiment with solubles. Chemical analyses presently available show that the behavior of the test organisms could have been affected by (1) hydrocarbons from the oil slick, (2) changes in the composition and concentration of the lipids other than the hydrocarbons, (3) influx of products of the biochemical oxidation of oil by microorganisms. However, analysis of lobster behavior showed no changes caused by this experiment; only in the experiment with whole crude oil mixed with sea water could behavior changes be measured.

B. Behavior Changes

In order of significance, the following behavior units changed after the lobsters were exposed to whole crude oil:

Behavior Unit	Direction of Change	X^2	Significance
1. Crude, Waiting Phase	increase	12.6	.001
2. Crude, Antennule Wave	increase	9.7	.005
3. Crude, Antenna Wave	increase	8.5	.005
4. Crude, Fan Slow	increase	8.5	.005
5. Crude, Beat Fast	decrease	3.1	.100

Exposure to the water soluble fraction of crude oil did not affect the lobsters' behavior in a way that could be measured by our methods.

The changes in behavior observed after exposure to whole crude oil may be summarized as changes in water chemistry sensing movements: 1) Slow rates of gill bailers increased, which means that less water was passed over the gills and around the lobsters' anterior end; 2) Fast antennule rates decreased slightly, which means that the lobster "sniffed" (to use a higher vertebrate analog) less intensely. Both results indicate that there is a tendency for an increase in slow fanning and beating movements. Finally, 3) Antennae and antennules moved more, which may be the equivalent of an increase in head movements in higher vertebrates, or more "looking around". The increase in antenna and antennule waves may compensate for the decrease in gill bailer and antennule beating rates; or, in vertebrate terms, head movements took over from sniffing in the presence of oil in the water.

Both phases of feeding behavior may involve sensory perception. The first or alerting phase, in which the animal becomes aware of a chemical stimulus, is probably determined by threshold perception of the chemical cues, most likely by antennular aesthetasc hairs of the sense of smell. The third or searching phase, in which the food is localized, probably involves chemical and tactile senses in the detection of odor gradients and water currents. The second or waiting phase, however, may or may not be under sensory control

One explanation for the doubling of the waiting phase is that the animal is still capable of perceiving chemical stimuli in its environment, but that the ability to interpret the stimuli as food is impaired by direct damage to the sensory receptor system. However, since no major morphological changes took p! .e in odor receptors, the aesthetasc hairs of the antennules, as evidenced by light and electron microscopy, sensory disruption probably did not occur at this level of oil exposure.

Discounting sensory damage, the waiting phase can be described as a period in which the animal must build up motivation (i.e., sufficient attracting stimulation) to leave the sheltered corner position and go out in search of food, the chemical presence of which is presumably known after alerting. Highly motivated animals (i.e., very hungry ones that are under little stress from ambient light, the presence of the observer, oil or any other environmental factor) become alerted and soon run off to find the food. In extreme cases, this phase is reduced to zero. Since all animals were kept hungry, a time increase in this phase must be due to the one environmental stress not held constant; oil. One might say that oil produces a bad taste (or odor) in the water which competes negatively with the attractive properties of the food odor. This competing effect of sensory inputs may take place at the receptor sites or, probably, in more central parts of the nervous system.

There also remains the possibility that oil interferes directly with the molecular properties of the food stimulus, changing its odor characteristics. This would result in difficulty in recognizing the chemical stimulus as food,hence a longer waiting time.

All explanations are based on the premise that less food stimulation reaches the receptors or that more food stimulation is necessary to offset the ill effects ("bad taste") of oil stimulation, Either way, the waiting time increases to allow for motivation (here, level of positive chemotactic stimulation) to build up. It may be this same "bad taste" effect that changes the water quality sensing movements of the lobsters' behavior in general. The possibility must be pointed out that the change in water quality sensing movements could directly affect the length of the waiting phase in feeding behavior.

It has been suggested that the changing chemical composition of degrading oil may have different levels of toxicity for marine life (Blumer, 1972). The small number of tests in relation to the individuality of the lobsters' behavior made it impossible to analyze, on a day by day basis, the oil effect corresponding with the chemical analyses. However, if a particular day and, therefore, a particular composition of the degrading oil had caused a uniform spectacular change in behavior, it would have been apparent in the results. Thus, at this exposure level, no major differences could be seen in the effects of oil degrading over a five day period.

In summary, the results show that crude oil, when mixed in small quantities (1 ml in 100 l) in sea water, has immediate, measurable effects on the behavior of adult male and female lobsters over a five day period. Changes in water quality sensing movements were observed and the time required to find food, even when hungry, was increased. Sensory damages by oil at these low levels seems unlikely from the results of behavior and microscopy, but interference with chemical communication nevertheless takes place when oil represents a negative

stimulus reducint the attraction of food. Long term effects and recovery were not studied. The experiments confirm that the low boiling fraction of oil rather rapidly disappears from sea water and that the higher boiling fraction increases with time in the water column. A correlation of behavior effects with the changing composition of degrading oil could not be made.

SECTION VII

ACKNOWLEDGMENTS

We want to express our great appreciation to Dr. John Farrington and Bruce Tripp of the Woods Hole Oceanographic Institute who did the chemical analyses of the water soluble fraction and crude oil-water mixture, respectively. Without their help this study would have lost much of its value.

We also want to thank Dr. Melbourne Carriker of the Marine Biological Laboratory at Woods Hole for the generous use of his excellent light microscopy equipment and Dr. Susumu Honjo and Joanne Antanavage of W.H.O.I. for their assistance in the electron microscopy.

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SECTION VIII

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SECTION IX

PENDING PUBLICATION

Atema, J. and L. Stein. Sublethal Effects of Crude Oil on Feeding Behavior in the lobster, Homarus americanus. Marine Behavior and Physiology, submitted.

SECTION X

APPENDICES

App. Table I: Complete list of behavior units (Homarus americanus)

App. Table IIa: Frequency of occurrence of selected behavior units

App. Table IIb: Frequency of occurrence of the behavior units "Beat", "Fan" and "Claw" presented in raw scores and normalized scores (%)

App. Table III: Duration of Phases in Feeding Behavior

APPENDIX TABLE I

COMPLETE LIST OF BEHAVIOR UNITS (Homarus americanus)

Name	Definition	Code
Beat	Beating of antennules at the following rates:	
Slow	0-60 bpm	aaS
Moderate	60-120 bpm	aaM
Fast	$\geq$ 120 bpm	aaF
Wipe	wiping of an antennule with 3d maxillipeds	aw
Antennule position 1-5	1↑; 2↗; 3→; 4↘; 5↓	aa^{1}; etc.
Antennule wave	change in antennule position	aT
Antennule point	movement of antennules in direction of stimulus	aP
Antenna wave	slow sweep of an antenna	aW
Antenna wipe	wiping of an antenna with 3d maxillipeds	Aw
Antenna position		
normal	in front	AAn
up	straight up	AAu
folded	folded back	AAf
back	between up and folded	AAb
Antenna feel	quick, successive movements of antennae over object	F
Antenna touch	antenna touching object one time	AT
Antenna point	movement of antenna in direction of stimulus	AP
Fan	Fanning of exopodites of 3rd maxillipeds; rates:	
Slow	0-40 bpm	exo S
Moderate	90-180 bpm	exo M
Fast	$\geq$ 190 bpm	exo F
Maxilliped movement	swaying of 3d maxillipeds	mm
Maxilliped rub	moving 3d maxillipeds against each other	Ru
Maxilliped extended	stretching of maxilliped down toward substrate	Me

Name	Definition	Code
Claw position 1	claw closed	S_1 or C_1
Claw position 2	claw ½ open	S_2 or C_2
Claw position 3	claw full open	S_3 or C_3
Claws		
Up	claw raised above eye level	S^1, etc.
Level	claw raised but below eye level	S1
Down	claw on substrate	S_1
Claw spread	claws raised and spread	ClS
Stretch claws	extend claws in front of body	St.C
Withdraw crusher	bring crusher claw close to body	WCr
Withdraw seizer	bring seizer claw close to body	WSe
Shield	Keep claws closed in front, passive	Sd
Body position		
low on legs	body close to substrate	lo legs
medium on legs	body partially raised off substrate	M legs
high on legs	body raised high	H legs
Crouch	sit very low on legs and body fairly compact	Cro
Tail position 1-5	1–; 2⌐; 3⌐; 4⌐; 5⊃;	T_1, etc.
Tail arch	curling tail upward	Arch
Tail hump	raising abdomen while tail is down	Hump
Defensive posture	Tail position 5; body raised, claws 2-3 and up	Def
Move	any undirected movement of the body	GBM
Walk	walk	W

Name	Definition	Code
Slow walk	slow walk	Slo W
Stop walk	stop walk	Stp W
Run	fast walk	Run
Reverse	walk backwards	Rev
Stop reverse	stop walk backwards	Stp Rev
Walk sideways	forward and lateral walk at the same time	WS
Approach	forward movement directed toward stimulus	app
Climb	raising of body against side of rocks, walls, etc. to a vertical position	Cl
Descend	climb down	D
Reverse descend	climb down backwards	RD
Raise rear	tail up on wall, rock etc. (describe)	RR
Fall	free fall descend	FD
Pounce	sudden lowering of body usually accompanied by feeding behavior	Pounce
Roll	lateral rotation of body	roll
Enter hole forward	crawl into hole forward (denote which hole)	HF
Enter hole reverse	crawl into hole tail first	H rev.
Turn	change direction of body	T
Rake	back and forth movement of one or more walking legs across the substrate while body is still	
I	slow rake	R I
II	moderate rake	R II
III	fast rake	R III
Scoop	lifting of walking legs from substrate into mouth	sc

Name	Definition	Code
Pinch	opening & closing dactyls of walking legs	pin
Poke	poke into substrate with legs	Po
Dig	moving substrate with legs	Dig
Bulldoze	push gravel with maxillipeds and/or claws	BD
Lunge	fast extension of claws	lunge
Snap	quick opening & closing of seizer claw	snap
Swimmeret wave	beating of swimmerets	sw
Stop swimmeret wave	stop beating of swimmerets	stp sw
Groom	picking, rubbing, scratching parts of body with the walking legs	GR
antenna		AA
antennules		aa
swimmerets		sw
carapace		car
rostrum		ros
eye		eye
claws		claws
legs		legs
tail		tail
Stop groom		stp Gr

Name	Definition	Code
AGGRESSIVE		
Being pulled	while in claw lock, being maneuvered by other animal forwards	puld
Being pushed	in encounter, being maneuvered by other other animal backwards	pud
Claw lock	handshake position of animals while in face off - crusher - crusher only	CL
Engage	maneuvering of claws to get into claw lock	En
Face off	face to face confrontation of two animals within one body length	FO
Grab	claw placed around part of other animal and bear down, including crusher-seizer hold	G
Jab	poking at other animal's body or claws with own claws	ja
Positioning	first stages of maneuvering in claw lock	Pn
Pull	while in claw lock, maneuvering other animal	pul
Push crusher	with crusher, push other animal. If not in claw lock, push will be with back of claw	PuC
Push seizer	same as above but with seizer	PuS
Rip	bearing down with claws and quick jerking of body--high intensity	Ri
Low rip	low intensity Rip	loRi
Shake	while in claw lock, movement of claw that is locked toward other animal and back toward self (slow moving)	Sh
Roll animal over	while in claw lock, due to strength, turning other animal on its side	RAO
Swat	swinging of seizer toward other animal (like a right hook)	Swat
Release	letting go of claw lock	Rel
On Guard	one claw raised and extended and other claw close to body and down	OG
MATING		
Dismount	walking away from mount	DisM
Ejaculate	thrust of abdomen of male while mating	ejac
Limp	describes submissive animal when being mated--no resistance	Li
Mount		
I	beginning stages--claws and/or maxillipeds on another animal	M I
II	1/2 way on other animal	M II
III	fully on other animal	M III
Resisting	opposite of Limp	Res
Turn animal over	after mounting, turning the submissive animal over	TAO

APPENDIX TABLE IIa

Frequency of occurrence of selected behavior units

Unit: Crude, Antenna Wave

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	4	5	2	2	5	14	14	4	16	10
2	16	14	5	3	19	8	28	29	29	7
3	8	9	3	1	22	26	22	36	32	29
4	5	3	1	6	11	17	7	9	9	3
5	14	9	0	1	2	10	1	5	0	1
6	2	8	0	0	6	10	14	6	6	1
7	10	8	2	5	18	2	5	20	9	12

Unit: Crude, Antennule Wave

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	0	0	0	2	0	1	0	1	0	1
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	6	1
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	3	0	0	1
6	0	4	1	0	1	2	9	3	2	3
7	0	0	0	0	0	1	0	0	1	1

Unit: Crude, Wipe

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	14	11	10	2	3	7	7	13	8	6
2	3	13	0	13	0	5	11	7	5	3
3	7	8	3	3	6	1	12	14	4	4
4	8	38	1	5	2	1	6	4	0	8
5	3	13	4	15	6	1	3	3	0	2
6	3	7	0	10	21	8	1	5	3	3
7	8	10	2	0	6	7	2	7	4	5

APPENDIX TABLE IIa continued

Unit: Crude, Rake

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	14	8	5	0	5	0	1	12	11	8
2	0	0	2	0	0	0	5	2	2	0
3	0	15	0	0	0	0	0	3	5	1
4	0	1	0	0	0	1	1	7	1	4
5	3	9	8	3	1	3	0	9	12	8
6	2	5	2	0	16	1	0	0	0	0
7	1	1	1	0	0	0	2	7	0	7

Unit: Crude, Scoop

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	14	10	4	0	0	0	8	7	10	12
2	0	0	0	0	0	0	13	1	0	0
3	0	53	0	0	0	0	1	4	0	3
4	0	0	0	0	0	0	0	1	0	0
5	2	18	8	0	0	0	0	3	0	0
6	0	0	0	0	9	0	0	0	0	0
7	0	0	0	0	0	0	0	2	0	4

Unit: Crude, Move

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	1	3	0	1	6	6	6	3	5	5
2	3	1	0	3	5	2	3	5	6	3
3	2	3	0	0	4	4	4	10	3	7
4	1	4	3	2	3	4	2	3	3	3
5	3	4	2	0	2	2	3	2	0	1
6	1	4	1	3	7	4	1	1	3	7
7	3	1	3	1	4	2	2	3	1	3

APPENDIX TABLE IIa cont.

Unit: Crude, Climb

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	5	2	7	0	2	0	0	0	2	3
2	0	0	2	0	0	0	4	5	0	1
3	0	0	3	0	0	0	0	0	0	0
4	0	1	0	0	3	0	2	0	1	0
5	4	0	0	0	0	2	0	4	0	0
6	0	0	1	0	1	0	0	0	0	0
7	0	0	0	0	1	0	0	0	0	0

Unit: Crude, Groom

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	15	20	4	0	2	0	0	19	8	10
2	0	2	0	3	0	0	1	2	0	0
3	0	0	0	0	0	0	0	0	4	0
4	1	0	2	0	0	0	0	0	8	0
5	0	3	1	0	0	6	0	1	0	0
6	1	0	1	0	8	1	1	0	0	0
7	2	3	0	0	0	0	0	4	0	0

APPENDIX TABLE IIa cont.

Unit: Soluble, Antenna Wave

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	15	5	6	2	3	11	3	11	5	14
2	30	27	31	22	12	25	43	8	13	49
3	33	19	15	14	21	24	24	6	3	3
4	10	16	23	15	7	5	14	14	30	13
5	15	41	4	20	7	7	10	6	16	17
6	20	3	17	17	3	18	28	18	24	5
7	33	15	6	13	20	15	17	13	16	15

Unit: Soluble, Antennule Wave

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	5	4	1	4	0	2	1	3	1	0
2	2	3	4	6	0	3	0	1	2	1
3	0	6	4	8	4	3	2	1	2	6
4	2	2	0	2	1	2	3	2	2	0
5	0	5	15	0	0	0	10	2	2	1
6	5	3	6	3	3	6	2	6	3	5
7	1	2	6	0	2	0	3	2	5	3

APPENDIX TABLE IIa cont.

Unit: Soluble, Wipe

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	2	3	5	0	3	3	2	4	5	2
2	6	3	5	5	7	7	3	4	0	6
3	3	7	10	5	6	6	8	5	5	3
4	2	9	2	5	4	6	12	7	2	6
5	2	3	3	9	4	3	0	3	1	4
6	6	0	1	2	5	2	3	4	4	5
7	2	23	3	13	3	23	3	11	5	3

Unit: Soluble, Rab

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	0	3	2	0	2	3	3	0	1	1
2	0	0	0	2	2	5	0	5	0	3
3	5	4	7	1	6	1	0	3	1	10
4	1	12	4	2	0	7	4	12	0	10
5	0	3	1	0	0	0	0	3	3	0
6	1	0	0	0	1	3	5	3	8	6
7	1	0	4	2	2	5	3	2	2	0

APPENDIX TABLE IIa cont.

Unit: Soluble, Scoop

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	4	0	5	0	0	2
2	0	0	0	0	0	6	0	0	0	1
3	1	1	0	0	0	1	0	0	2	3
4	0	27	0	3	0	2	0	13	0	1
5	0	11	0	0	0	0	0	0	0	0
6	0	0	0	0	2	0	0	0	0	0
7	0	2	1	1	1	3	0	7	2	0

Unit: Soluble, Move

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	0	4	1	0	3	2	1	3	3	1
2	0	1	2	0	1	2	0	2	1	1
3	0	1	1	3	1	3	1	2	3	0
4	5	4	1	1	5	2	3	2	2	4
5	2	0	1	1	1	1	0	1	2	1
6	3	0	0	1	0	2	2	0	4	1
7	1	3	0	0	3	5	2	3	2	1

Unit: Soluble, Climb

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	0	0	2	1
2	0	0	0	1	1	0	14	0	0	1
3	10	3	5	4	8	2	2	0	0	0
4	0	0	3	0	0	0	0	0	0	0
5	0	4	0	0	0	0	0	0	0	0
6	0	0	0	0	0	1	1	0	0	0
7	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE IIa cont.

Unit: Soluble, Groom

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	0	1	0	0	7	0	13	8	0	1
2	1	2	0	8	0	5	16	1	1	0
3	0	9	0	3	2	0	30	33	22	16
4	6	25	1	10	0	0	3	0	0	0
5	4	12	0	0	1	0	0	9	13	0
6	3	0	0	0	0	0	0	0	0	0
7	0	0	1	0	0	1	0	3	0	0

APPENDIX TABLE IIb

Unit: Crude, Beat

Animal Number		Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	Slow	0	2	2	5	2	4	2	3	3	2
	%	0	25	40	83	50	50	100	38	43	33
	Moderate	1	5	3	1	2	4	0	4	3	3
	%	12	63	60	17	50	50	0	50	43	50
	Fast	7	1	0	0	0	0	0	1	1	1
	%	38	12	0	0	0	0	0	12	14	17
2	Slow	5	2	4	1	0	1	0	3	5	4
	%	50	15	80	20	0	12	0	23	38	40
	Moderate	5	10	1	4	6	7	4	8	8	6
	%	50	77	20	80	67	88	57	62	62	60
	Fast	0	1	0	0	3	0	3	2	0	0
	%	0	8	0	0	33	0	43	15	0	0
3	Slow	6	3	3	3	4	1	1	1	3	4
	%	67	100	43	50	66	10	20	14	43	44
	Moderate	3	0	1	2	1	8	4	3	4	5
	%	33	0	14	33	17	80	80	43	57	56
	Fast	0	0	3	1	1	1	0	3	0	0
	%	0	0	43	17	17	10	0	43	0	0
4	Slow	1	4	5	3	5	4	5	6	3	5
	%	17	57	71	75	46	40	63	86	50	100
	Moderate	5	2	2	1	4	5	3	1	3	0
	%	83	29	29	25	36	50	37	14	50	0
	Fast	0	1	0	0	2	1	0	0	0	0
	%	0	14	0	0	18	10	0	0	0	0
5	Slow	2	4	3	4	5	2	6	3	3	2
	%	22	50	75	100	62	40	67	34	75	100
	Moderate	4	3	0	0	3	2	2	4	1	0
	%	44	38	0	0	38	40	33	44	25	0
	Fast	3	1	1	0	0	1	0	2	0	0
	%	34	12	25	0	0	20	0	22	0	0
6	Slow	2	4	1	4	5	3	7	4	4	8
	%	40	33	100	50	72	43	88	80	57	80
	Moderate	2	8	0	4	1	4	1	1	3	2
	%	40	67	0	50	14	57	12	20	43	20

APPENDIX TABLE IIb cont.

Unit: Crude, Beat

Animal Number		Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
	Fast	1	0	0	0	1	0	0	0	0	0
	%	20	0	0	0	14	0	0	0	0	0
7	Slow	4	3	4	3	7	5	5	6	2	5
	%	50	75	67	38	70	83	100	86	67	83
	Moderate	4	1	2	5	2	1	0	1	1	1
	%	50	25	33	62	20	17	0	14	33	17
	Fast	0	0	0	0	1	0	0	0	0	0
	%	0	0	0	0	10	0	0	0	0	0

APPENDIX TABLE IIb cont.

Unit: Crude, Fan

Animal Number		Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	Slow	2	3	0	0	2	3	2	1	3	1
	%	100	100	0	0	67	100	100	100	100	100
	Moderate	0	0	0	0	1	0	0	0	0	0
	%	0	0	0	0	33	0	0	0	0	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
2	Slow	1	3	0	0	0	1	0	0	3	1
	%	100	60	0	0	0	100	0	0	60	100
	Moderate	0	2	0	0	0	0	0	0	2	0
	%	0	40	0	0	0	0	0	0	40	0
	Fast	0	0	2	0	0	0	1	1	0	0
	%	0	0	100	0	0	0	100	100	0	0
3	Slow	2	3	0	0	4	1	1	3	3	1
	%	100	100	0	0	100	100	100	50	100	50
	Moderate	0	0	0	0	0	0	0	3	0	1
	%	0	0	0	0	0	0	0	50	0	50
	Fast	0	0	1	0	0	0	0	0	0	0
	%	0	0	100	0	0	0	0	0	0	0
4	Slow	1	3	0	0	1	0	2	1	1	3
	%	100	75	0	0	100	0	100	100	100	100
	Moderate	0	1	0	0	0	0	0	0	0	0
	%	0	25	0	0	0	0	0	0	0	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
5	Slow	0	4	0	0	3	1	2	1	2	2
	%	0	80	0	0	100	100	100	50	100	100
	Moderate	1	1	0	0	0	0	0	1	0	0
	%	50	20	0	0	0	0	0	50	0	0
	Fast	1	0	0	0	0	0	0	0	0	0
	%	50	0	0	0	0	0	0	0	0	0
6	Slow	0	2	0	0	3	1	0	2	1	1
	%	0	100	0	0	100	100	0	100	100	100
	Moderate	0	0	0	1	0	0	0	0	0	0
	%	0	0	0	100	0	0	0	0	0	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
7	Slow	1	2	0	0	1	2	0	3	1	2
	%	100	100	0	0	100	100	0	100	100	100
	Moderate	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE IIb cont.

Unit: Crude, Claw

Animal Number	Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1. Position I	7	4	2	0	0	0	0	0	2	0
%	70	40	33	0	0	0	0	0	13	0
Position II	3	6	4	2	4	2	4	5	13	12
%	30	60	67	100	100	100	100	100	87	100
Position III	0	0	0	0	0	0	0	0	0	0
%	0	0	0	0	0	0	0	0	0	0
2. Position I	0	4	2	0	0	0	4	1	0	0
%	0	67	100	0	0	0	50	6	0	0
Position II	3	2	0	4	4	0	4	15	6	2
%	75	33	0	100	100	0	50	94	100	100
Position III	1	0	0	0	0	0	0	0	0	0
%	25	0	0	0	0	0	0	0	0	0
3. Position I	0	0	0	0	0	0	0	0	1	0
%	0	0	0	0	0	0	0	0	50	0
Position II	3	1	2	0	4	0	3	4	1	4
%	75	50	100	0	67	0	75	67	50	100
Position III	1	1	0	0	2	0	1	2	0	0
%	25	50	0	0	33	0	25	33	0	0
4. Position I	0	2	1	0	0	0	4	0	1	0
%	0	100	25	0	0	0	67	0	25	0
Position II	2	0	3	2	4	2	2	4	2	2
%	100	0	75	100	100	100	33	100	50	100
Position III	0	0	0	0	0	0	0	0	1	0
%	0	0	0	0	0	0	0	0	25	0
5. Position I	3	2	0	0	0	2	0	2	0	0
%	75	40	0	0	0	33	0	33	0	0
Position II	1	3	2	3	2	4	2	4	2	2
%	25	60	100	75	100	67	100	67	100	100
Position III	0	0	0	1	0	0	0	0	0	0
%	0	0	0	25	0	0	0	0	0	0
6. Position I	0	0	2	0	2	0	0	0	0	0
%	0	0	100	0	50	0	0	0	0	0
Position II	0	0	0	2	2	0	0	0	0	0
%	0	0	0	100	50	0	0	0	0	0
Position III	0	0	0	0	0	0	0	0	0	0
%	0	0	0	0	0	0	0	0	0	0
7. Position I	0	0	0	0	2	0	0	1	0	0
%	0	0	0	0	50	0	0	25	0	0
Position II	4	0	1	2	2	2	0	3	2	3
%	100	0	50	100	50	100	0	75	100	75
Position III	0	0	1	0	0	0	0	0	0	1
%	0	0	50	0	0	0	0	0	0	25

APPENDIX TABLE IIb cont.

Unit: Soluble, Beat

Animal Number		Days pre-oil					Days post-oil				
		1	2	3	4	5	6	7	8	9	10
1	Slow	10	7	10	10	5	6	6	8	5	11
	%	77	64	91	100	83	33	60	67	24	79
	Moderate	2	4	1	0	1	11	4	4	11	3
	%	15	36	9	0	17	61	40	33	52	21
	Fast	1	0	0	0	0	1	0	0	5	0
	%	8	0	0	0	0	6	0	0	24	0
2	Slow	4	6	7	9	8	12	6	3	15	5
	%	20	21	44	69	36	57	43	20	100	25
	Moderate	11	13	8	3	10	8	6	12	0	12
	%	55	45	50	23	45	38	43	80	0	60
	Fast	5	10	1	1	4	1	2	0	0	3
	%	25	34	6	8	18	5	14	0	0	15
3	Slow	10	8	9	11	6	8	6	6	11	13
	%	50	50	39	55	24	33	35	60	92	93
	Moderate	5	6	9	7	15	16	7	4	1	1
	%	25	38	39	35	60	67	41	40	8	7
	Fast	5	2	5	2	4	0	4	0	0	0
	%	25	13	22	10	16	0	24	0	0	0
4	Slow	5	10	5	11	11	11	5	12	7	11
	%	26	63	21	65	58	58	25	55	24	65
	Moderate	14	6	18	6	8	8	15	10	21	6
	%	74	37	75	35	42	42	75	45	72	35
	Fast	0	0	1	0	0	0	0	0	1	0
	%	0	0	4	0	0	0	0	0	3	0
5	Slow	11	9	17	12	10	14	11	10	20	14
	%	55	50	100	57	77	100	100	100	95	82
	Moderate	9	7	0	9	3	0	0	0	1	3
	%	45	39	0	43	23	0	0	0	5	18
	Fast	0	2	0	0	0	0	0	0	0	0
	%	0	11	0	0	0	0	0	0	0	0
6	Slow	11	10	12	9	9	11	9	13	10	6
	%	42	100	57	38	50	55	43	100	53	50
	Moderate	14	0	9	15	9	9	12	0	9	6
	%	54	0	43	62	50	45	57	0	47	50
	Fast	1	0	0	0	0	0	0	0	0	0
	%	4	0	0	0	0	0	0	0	0	0
7	Slow	16	12	16	10	11	4	4	10	14	9
	%	72	80	84	77	92	25	20	100	88	53
	Moderate	6	3	3	3	1	12	16	0	2	8
	%	28	20	16	23	8	75	80	0	12	47
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE IIb cont.

Unit: Soluble, Fan

Animal Number		Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
1	Slow	4	7	10	0	3	7	5	8	2	9
	%	100	100	100	0	50	100	83	100	50	100
	Moderate	0	0	0	0	3	0	1	0	2	0
	%	0	0	0	0	50	0	17	0	50	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
2	Slow	2	5	5	3	1	6	4	0	2	6
	%	40	83	100	100	100	86	33	0	100	100
	Moderate	3	1	0	0	0	1	4	1	0	0
	%	60	17	0	0	0	14	33	100	0	0
	Fast	0	0	0	0	0	0	4	0	0	0
	%	0	0	0	0	0	0	33	0	0	0
3	Slow	7	4	6	5	3	4	5	9	10	8
	%	41	100	55	83	43	100	100	69	100	100
	Moderate	3	0	5	1	3	0	0	4	0	0
	%	18	0	45	17	43	0	0	31	0	0
	Fast	7	0	0	0	1	0	0	0	0	0
	%	41	0	0	0	14	0	0	0	0	0
4	Slow	7	3	7	8	9	10	3	10	4	11
	%	100	100	64	100	100	100	100	100	44	92
	Moderate	0	0	3	0	0	0	0	0	5	1
	%	0	0	27	0	0	0	0	0	56	8
	Fast	0	0	1	0	0	0	0	0	0	0
	%	0	0	9	0	0	0	0	0	0	0
5	Slow	8	0	0	0	11	0	10	10	6	6
	%	100	0	0	0	100	0	100	77	50	100
	Moderate	0	6	6	0	0	0	0	3	6	0
	%	0	50	100	0	0	0	0	23	50	0
	Fast	0	6	0	0	0	0	0	0	0	0
	%	0	50	0	0	0	0	0	0	0	0
6	Slow	5	0	8	0	10	11	12	0	10	10
	%	83	0	100	0	100	100	80	0	91	100
	Moderate	1	0	0	5	0	0	3	0	1	0

APPENDIX TABLE IIb cont.

Unit: Soluble, Fan

Animal Number		Days pre-oil 1	2	3	4	5	Days post-oil 6	7	8	9	10
	%	17	0	0	100	0	0	20	0	9	0
	Fast	0	0	0	0	0	0	0	0	0	0
	%	0	0	0	0	0	0	0	0	0	0
7	Slow	7	8	8	11	8	5	8	10	12	6
	%	100	89	67	69	100	38	47	100	80	43
	Moderate	0	1	4	5	0	8	11	0	3	7
	%	0	11	33	31	0	62	53	0	20	50
	Fast	0	0	0	0	0	0	0	0	0	1
	%	0	0	0	0	0	0	0	0	0	7

APPENDIX TABLE III - DURATION OF PHASES

IN FEEDING BEHAVIOR

CRUDE, ALTERTING PHASE

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	:25	:15	:11	:18	:47	:27	:20	:05	:05	:07
2	:17	:22	:20	:15	:06	:23	:17	:25	:22	:26
3	:10	:21	:25	:12	:17	:05	:11	:04	:07	:10
4	:17	:06	:15	:45	:13	:40	:30	:18	:15	:08
5	:20	:15	:25	:30	:07	:20	:30	:15	:10	:31
6	:28	:20	:15	:20	:09	:35	:38	:12	:20	:15
7	:40	:10	:35	:25	:35	:20	:15	:17	:28	:30

APPENDIX TABLE III cont.

CRUDE, WAITING PHASE

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	:12	:10	:12	:00	:00	:78	:55	:10	:12	:10
2	:21	:17	:00	:65	:72	:45	:46	:40	:58	:60
3	:07	:04	:00	:00	:02	:03	:22	:11	:10	:15
4	:04	:08	:07	:25	:54	:35	:25	:44	:45	:70
5	:20	:53	:00	:00	:11	:03	:22	:13	:15	:08
6	:05	:05	:08	:18	:14	:27	:17	:10	:92	:13
7	:07	:40	:20	:15	:65	:98	:130	:30	:47	:47

APPENDIX TABLE III cont.

CRUDE, SEARCHING PHASE

Animal Number	Days pre-oil						Days post-oil			
	1	2	3	4	5	6	7	8	9	10
1	:06	:04	:12	:04	:09	:09	:07	:15	:03	:03
2	:38	:10	:12	:12	:24	:07	:18	:12	:07	:06
3	:15	:10	:25	:07	:22	:29	:12	:10	:03	:30
4	:45	:61	:38	:08	:37	:13	:35	:11	:26	:57
5	:07	:13	:12	:33	:08	:12	:09	:06	:08	:04
6	:07	:10	:04	:08	:05	:07	:06	:04	:08	:07
7	:09	:08	:05	:22	:16	:34	:06	:11	:05	:05

APPENDIX TABLE III cont.

SOLUBLE, ALERTING PHASE

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	:25	:08	:28	:40	:15	:105	:24	:20	:22	:22
2	:21	:18	:27	:14	:07	:65	:25	:35	:26	:20
3	:24	:30	:50	:10	:35	:25	:12	:13	:22	:21
4	:28	:14	:15	:08	:15	:15	:12	:20	:07	:07
5	:18	:27	:29	:07	:14	:12	:32	:14	:15	:08
6	:23	:13	:15	:15	:12	:15	:10	:20	:13	:07
7	:28	:26	:26	:60	:36	:09	:06	:13	:12	:13

APPENDIX TABLE III cont.

SOLUBLE, WAITING PHASE

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	:42	:58	:32	:45	:15	:100	:08	:26	:38	:14
2	:25	:10	:43	:80	:13	:84	:68	:101	:39	:30
3	:08	:26	:26	:23	:58	:18	:13	:17	:03	:05
4	:12	:20	:10	:06	:04	:40	:21	:46	:34	:48
5	:17	:21	:09	:12	:02	:20	:19	:16	:14	:14
6	:17	:04	:21	:07	:11	:14	:07	:06	:19	:20
7	:16	:09	:33	:06	:15	:31	:46	:37	:40	:30

APPENDIX TABLE III cont.

SOLUBLE, SEARCHING PHASE

Animal Number	Days pre-oil					Days post-oil				
	1	2	3	4	5	6	7	8	9	10
1	:05	:04	:22	:04	:12	:87	:13	:20	:06	:09
2	:04	:09	:15	:17	:05	:15	:04	:07	:02	:08
3	:08	:13	:22	:07	:12	:07	:08	:08	:05	:07
4	:06	:05	:06	:06	:06	:11	:07	:08	:07	:08
5	:15	:31	:32	:05	:06	:06	:09	:04	:06	:08
6	:18	:13	:07	:15	:06	:13	:26	:08	:04	:14
7	:08	:09	:18	:15	:18	:07	:10	:07	:26	:28

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